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PREAMBLE

In Southeast Asia tropical diseases usually cause a great number of health problems with particular influence on the socioeconomic status of human resources. Our SMRL research physicians and scientists have striven forwards to discover the etiologic factors of these ailments and their complications.

The accomplishment of research studies form the scientific bases in solving many problems of the disease process. Undoubtedly further investigations are mandatory for the unsolved areas of the individual or group of diseases.

Apparently the research topics in this volume have been properly grouped into 5 categories : virus diseases, bacterial diseases, parasitic diseases, and the other two separate sections under the miscellaneous group and the malaria studies. The outcome of these experimental and clinical research studies are by all means contributory to the medical progress.

Thutchai Dhirathumrong.

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VIRUS DISEASES OF MAN AND ANIMALS

Dengue Infection at the Children's Hospital of Bangkok

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OBJECTIVE: To provide viral diagnostic and laboratory expertise to the Children's Hospital of Bangkok and to collect specimens for specialized dengue virus isolation and serology.

BACKGROUND: Dengue virus infections continue to be an annoying and potentially critical problem for military forces stationed in many tropical areas. Dengue infections are also a major cause of morbidity and mortality among children in Southeast Asia. As in previous years SEATO Medical Research Laboratory has collaborated with the Children's Hospital of Bangkok in the study of dengue hemorrhagic fever. This has been mutually beneficial allowing for improved patient care through diagnostic and laboratory work provided by SEATO Laboratory and allowing for the collection of specialized samples from dengue patients to allow for investigations of the pathogenesis and clinical expressions of this infection.

DESCRIPTION: Patients with a hospital admission diagnosis compatible with dengue infections (dengue hemorrhagic fever, dengue fever or undifferentiated fever) were selected from the infectious disease wards of the Bangkok Children's Hospital. A standardized chart of pertinent signs, symptoms and laboratory findings was instituted on each patient.

An attempt was made to collect blood on at least the day of diagnosis and on the third, fifth, fifteenth and thirtieth days after hospitalization. Blood was allowed to clot or was collected using heparinized tubes (~20 u heparin/ml blood). Studies were done on either serum or plasma. During the month of July and August plasma was removed from heparinized blood and the cellular components were separated using a dextran sedimentation technique. Peripheral blood leukocytes were used by Dr. Nyven J. Marchette, University of Hawaii, in an investigation of the occurrence and specificity of *in vitro* antibody production, and to study the phenomenon of permissive peripheral blood leukocytes previously identified in monkeys. Platelets and, in a few cases, leukocytes as well as plasma were submitted for virus isolation.

Virus isolation was performed using a direct and delayed plaque technique. Plasma was also inoculated into mosquitoes, both at SEATO Medical Research Laboratory and at the University of Hawaii, to test the mosquito isolation technique of Rosen *et al* (see elsewhere in this report). Sera or plasma were used for serology; hemagglutination inhibition (HI) tests were performed using suckling mouse brain antigens prepared from dengue 1 (Hawaii), dengue 2 (New Guinea C), dengue 3 (H–87), dengue 4 (H–241), Japanese Encephalitis (Nakayama) and Chikungunya (Ross). Sera were extracted with acetone and tested against eight units of antigen. All sera collected from one patient were tested simultaneously.

At the conclusion of hospitalization, a clinical discharge diagnosis was made and the severity of the illness was independently classified by clinicians in charge of the case.

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Grading of severity of dengue hemorrhagic fever used criteria established by one of us.

Grade I: Fever accompanied by non-specific constitutional symptoms. The only hemorrhagic manifestation is a positive tourniquet test.

Grade II: Fever and skin hemorrhage or other bleeding such as epistaxis or gingival hemorrhage.

Grade III: Circulatory failure manifested by weak, rapid pulse with narrowing of pulse pressure (less than 20 mm Hg) or hypotension (systolic pressure 90 mm Hg or less).

Grade IV: Moribund patients with undetectable blood pressure or pulse.

Following grading, isolation and serological data were used to identify those patients infected with dengue and to determine the type of antibody response. Patients were considered to have had a dengue infection if a four-fold rise in HI antibody titer to at least two of the group B antigens was found between the acute and convalescent sera or if convalescent antibody titers to at least two antigens equaled or exceeded 1: 640. Criteria for the identification of primary or secondary dengue have been previously reported. Patients with convalescent HI titers of 1 : 640 or more to at least two dengue antigens were considered to have secondary infections while those with convalescent HI titers of less than 1: 640 were considered to have primary infections. Where necessary to clarify the occurrence of a primary or secondary infection, plaque reduction neutralization by selected sera of appropriate Group A and Group B seed viruses were performed.

In a few cases immunoglobulin separations were performed using sucrose gradient ultracentrifugation. The original sera and the fractions obtained from the sucrose gradients were tested for IgM, IgG and B1C/B1A concentrations using radialdiffusion plates (Hyland Laboratories). Antibody contained in these fractions was assayed by hemagglutination inhibition with and without treatment with 2 mercaptoethanol (2 ME). The 2 ME treatment was used to reduce IgM antibody activity.

One hundred and thirty four patients with admission diagnoses compatible with dengue infection were seen on the ward. One hundred and twenty seven of these were adequately followed and 114 (90%) were diagnosed as dengue infection by viral isolation, by serological criteria or both. Sixteen strains of dengue virus were isolated by direct or delayed plaque technique from either the plasma or the cellular components from the blood of the 114 patients with evidence of dengue infection (Table 1). In some cases isolations were made from the cellular components only. This represents an isolation rate of 14%. Five strains were dengue 1, five were dengue 2, three were dengue 3 and three are as yet unclassified. No dengue 4 was identified in Bangkok in 1974. Further details of isolation by the direct and delayed plaque technique and the mosquito isolation procedure will be found elsewhere in this report.

Of the 114 patients diagnosed as dengue, 13 (11.4%) patients had low level antibody responses detected by HI and were considered primary dengue infections, 94 (84%) had high HI titers and were considered secondary infections. Of the latter, 10 patients exhibited high fixed titers and 84 showed rising titers. Seven (6%) patients died, usually before the fifth day of disease. As no convalescent sera could be collected in these cases, the patients could not be classified on serological grounds as having primary or secondary infections. Laboratory findings on the 107 patients on whom classification could be completed may be found in Table 2. Dengue hemorrhagic fever occurred in patients with either primary or secondary infections and the distribution of clinical grades was essentially similar to that found in previous studies of hospitalized patients with one major exception.

This year, close clinical observation allowed for the detection of shock in three patients (D74-77, 91 and 103) exhibiting an antibody pattern characteristic of primary dengue infection (Table 3).

These three patients were investigated further. Figure 1 is a flow diagram of the clinical course of one of them (D74-77). In this patient shock occurred in the morning of the sixth day at a time when the fever was subsiding. The rising hematocrit and the falling platelet counts occurred over a short period of time just prior to the onset of shock. HI antibody titers at this time were between <1:20 and 1:80

against dengue 1 virus and were lower against other dengue types. Complement factor three, as estimated from the B1C/B1A concentrations, was 74 mg% on day 5; it fell to 54 mg% on day 7 and rose slightly to 64 mg% by day 9. It was measured again on days 19 and 34 when levels had returned to 136 mg%; a value within the normal range of 143 $\pm$ 22 mg% reported by Hyland Laboratories. The clinical course of D74–77 was essentially similar to those of the other two cases investigated.

From one of these patients (D74–103) a dengue 3 virus was isolated. No virus was identified in the other two (D74–77 and 91). Plaque reduction neutralization tests of all four dengue types and Chikungunya were performed on acute and convalescent sera from each patient. The results are currently available from two of them (D74–77, 91) (Table 3). There was no appreciable antibody to any of the viruses in either of the acute sera obtained on the fifth day of disease. In the convalescent sera from both cases high titered antibody to dengue 1 was found. In one (D74–77), low level antibody was also found to dengue type 3 and to Chikungunya. This patient received several units of plasma which may have contained antibody to group A and B arboviruses and might have caused the serological findings.

In order to determine whether dengue specific IgM was produced in these patients as would be expected in primary infection, sucrose gradient ultracentrifugation was performed on convalescent sera (Table 4). IgM antibody was found in the second through the fourth sucrose fraction (35–31% sucrose) of each serum studied. Specific IgM (reduced by 2 ME) was found against all four dengue types when tested by HI. In D74–77 and 91 the highest titers were found against dengue 1. In a convalescent serum from a case of secondary dengue (identified by HI antibody titers $\geq$ 1:20480), which was centrifuged simultaneously, IgM antibody was again found in the second, third and fourth fraction. A low level of antibody activity was also found in these fractions against all four dengue types but it could not be reduced by 2 ME indicating that the antibody activity was possibly due to IgG contamination. In none of the sera tested in this manner was any 2 ME reducible antibody to Chikungunya detected in the IgM portion of the gradient. In the convalescent serum from D74–77, low level antibody to Chikungunya was found in the IgG portion of the gradient; this activity was not reduced by 2 ME.

Because of the association of shock in dengue with low complement levels, B1C/B1A concentrations were determined on the acute and convalescent sera of all three patients. In all cases the B1C/B1A concentrations were reduced to less than 50% of normal in the acute sera and in sera taken at the time of shock. The concentration increased to the normal range during convalescence. B1C/B1A levels in five patients with primary disease without shock were either in the normal range or only slightly depressed throughout the course of the disease.

DISCUSSION: As in previous years SEATO Medical Research Laboratory has provided laboratory and diagnostic support to the Children's Hospital of Bangkok in an attempt to delineate dengue infection in Bangkok. This year, through close clinical observation, shock was detected in three patients whose HI antiboby titers indicated a primary dengue infection. Efforts to firmly establish the nature of these infections are presently underway.

Table 1. Dengue Isolation by Direct and Delayed Plaque Technique – 1974

Patient	Source[a]	Plaque Technique[b]			Identification
		Direct	Delayed	Secondary Delayed	
D74–16	Plasma	11[c]	–	–	D2
D74–33	Plasma	28	–	–	D3
	Platelets	106	–	–	
D74–38	Plasma	16	–	–	D2
	Platelets	15	–	–	
D74–44	Plasma	7	–	–	D1
D74–61	Platelets	0	6	–	D1
D74–63	Plasma	0	22	–	D1
	Platelets	2	–	–	
D74–66	Platelets	0	TNTC[d]	–	D2
D74–74	Leukocytes	2	–	–	D2
D74–90	Plasma	0	72	–	?
D74–95	Plasma	63	–	–	D1
D74–103	Plasma	121	–	–	D3
D74–104	Plasma	0	0	66	?
D74–112	Plasma	118	–	–	D1
D74–137	Plasma	0	17	–	?
D74–150	Plasma	TNTC	–	–	D2
D74–151	Plasma	TNTC	–	–	D3

a – Isolation from plasma was attempted in every case.

b – Direct, Delayed and Second delayed plaque techniques were used; using 0.3 ml of plasma

c – Number of plaques counted.

d – TNTC = too numerous to count.

Table 2. Hemagglutination Inhibition Antibody Levels in Convalescent Sera from 107 Patients with Dengue Infection

Grade of Disease	Primary Infection Titer <1:640	Secondary Infection Tirer ≥1:640
UC[a]	0	1 (0.9%)
UF[b]	5 (4.7%)	8 (7.4%)
I & II	5 (4.7%)	50 (46.7%)
III	3 (2.8%)	29 (27.1%)
IV	0	6 (5.6%)
TOTAL	13 (12.1%)	94 (87.8%)

a. UC indicates that the patient was unclassified.
b. UF indicates undifferentiated fever.

Table 3. Hemagglutination Inhibition and Plaque Reduction Neutralization Tests of Selected Dengue Hemorrhagic Fever Patients Seen in 1974

Study Number	Day of Disease	Reciprocal Hemagglutination Inhibition Titer				Reciprocal Plaque Reduction Neutralization Titer				
		D1	D2	D3	D4	D1	D2	D3	D4	Chikk
D74–77	5	<20	<20	<20	<20	<20	<20	<20	~40	<10
	17	80	40	160	160	>1280	~40	~120	>40	~160
	36	40	20	40	40	>1280	~40	~230	≥40	~120
D74–91	5	40	<20	20	<20	<20	<20	<20	<20	ND
	11	160	40	80	160	<20	<20	<20	<20	ND
	67	160	80	80	160	500	<40	<40	<40	<10
D74–103	3	<20	<20	<20	<20	NC	NC	NC	NC	NC
	7	<20	20	40	<20	NC	NC	NC	NC	NC
	58	20	20	160	80	NC	NC	NC	NC	NC

ND = Not done
NC = Not complete

Table 4. IgM Antibody Titrations of Sera Taken from Selected Dengue Hemorrhagic Fever Patients Seen in 1974

Patient No.	Day of Disease	Sucrose Fraction	IgM* mg %	IgG* mg %	Reciprocal HI Antibody Titer							
					D 1***		D 2		D 3		D 4	
					B**	2ME	B	2ME	B	2ME	B	2ME
D74–77	9 (1725)	2	15	0	16	0	0	0	4	0	8	0
		3	24	0	32	0	4	0	16	0	8	0
		4	> 50	0	64	0	32	0	8	0	64	0
		5	20	0	16	0	8	0	8	0	16	0
D74–91	6 (1993)	2	20	0	32	0	0	0	8	0	4	0
		3	44	0	64	0	4	0	8	0	8	0
		4	7	0	32	0	0	0	4	0	8	0
		5	0	0	32	0	0	0	4	0	8	0
D74–103	7 (2112)	2	11	0	0	0	0	0	0	0	0	0
		3	> 50	0	0	0	0	0	32	4	0	0
		4	14	0	0	0	0	0	8	0	0	0
		5	0	4.5	0	0	0	0	0	0	0	0
D74–6	11 (1087)	2	18	0	8	8	8	4	16	16	8	4
		3	35	0	16	16	16	8	32	32	16	16
		4	12.5	0	64	32	16	16	64	64	32	32
		5	0	8.5	256	512	128	256	256	512	256	256

* Concentrations of IgM and IgG as detected by radialimmunodiffusion

** Sucrose gradient fractions pretreated with buffer

*** Sucrose gradient fractions pretreated with 2 mercaptoethanol

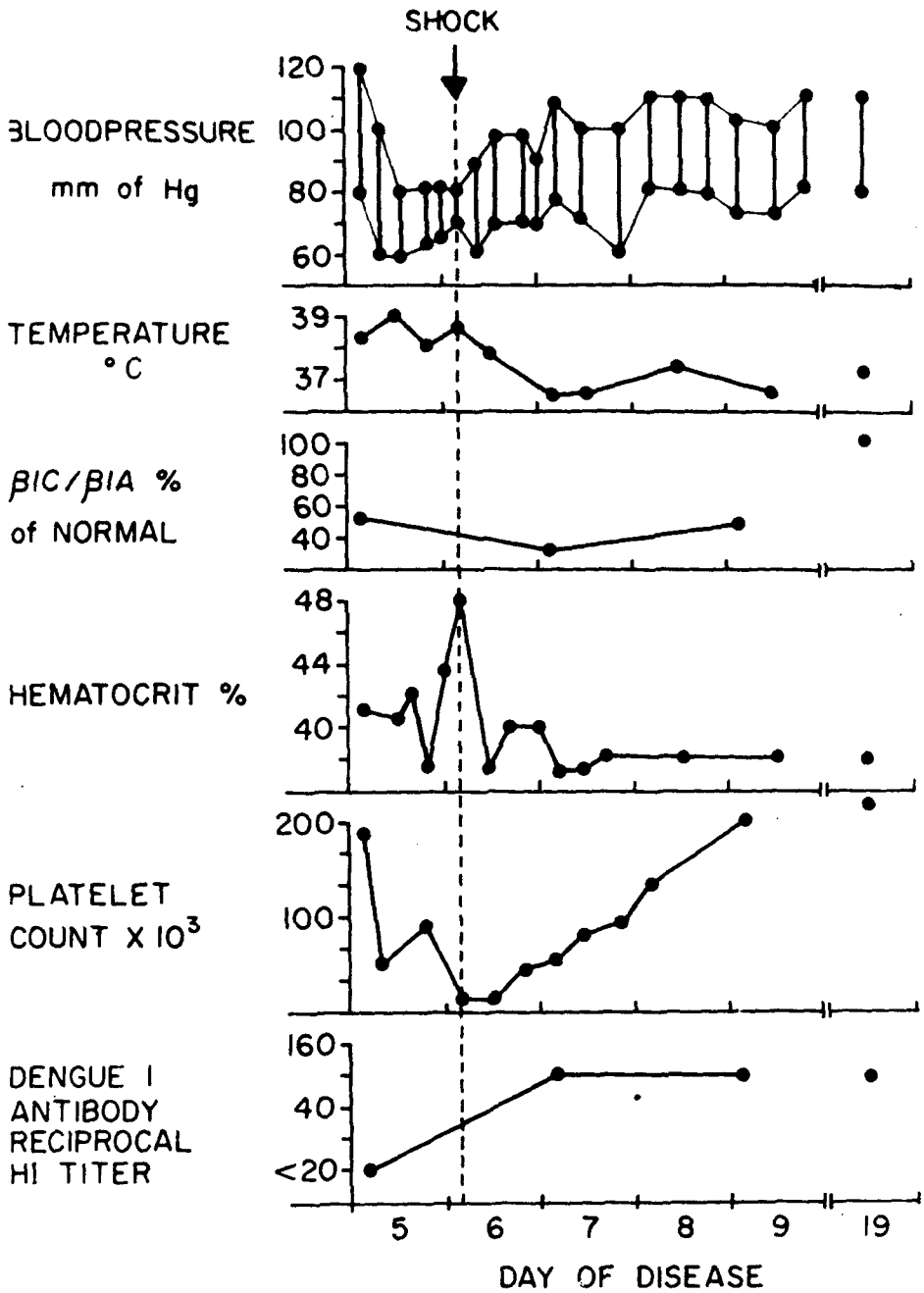

Figure 1 Diagram of the clinical course of patient D74-77 showing the relationship of several clinical and laboratory variables to the onset of shock.

Surveillance of Dengue Hemorrhagic Fever Cases in Thailand, 1973 and 1974

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OBJECTIVE: The purpose of this study is to confirm the clinically diagnosed dengue hemorrhagic fever (DHF) cases reported to the Ministry of Public Health by using a hemagglutination inhibition (HI) serum screening technique.

BACKGROUND: Dengue hemorrhagic fever remains a major infectious disease in every province and town of Thailand, manifested by high mortality and morbidity in children under 14 years old. The social and demographic features of Thailand make effective control of DHF a complex problem. This program is a long-range study to help in planning public health DHF control measures. An earlier report contains the results obtained from a study of acute and convalescent blood of clinical DHF cases (1). This report compares the results from the first two years of this surveillance program.

DESCRIPTION: In 1974, 70 provincial hospitals submitted acute and convalescent dried blood for testing compared to only 60 provinces in 1973. The methods for blood collection on filter paper discs and HI tests have been described (2).

PROGRESS: The localities of provinces and towns contributing to the study are shown in Figure 1. The total number of cases tested in 1974 was 2850, a 130% increase over the 1236 cases of 1973. At the same time, the total number of cases reported to the Ministry of Health was nearly the same for both years. It appeared that in 1974 more communities participated in the program and many provinces submitted more specimens for testing.

Dengue infections were confirmed for 491 patients in 1973 and 1042 in 1974, but the frequency of confirmation fell from 40% to 37% since many more negative specimens were also obtained during the second year (Table 1). Specimens from 178 patients (13%) were unsatisfactory for testing in 1973, because of insufficient blood, incomplete clinical information, unpaired blood specimens or laboratory technical problems; these specimens were considered undetermined. In 1974, only 17 patients (0.6%) fell into the undetermined category. The striking decrease in the number of patients with undetermined results also reflects improved operation of the surveillance system.

Figures 2 to 6 illustrate the frequency of positive dengue cases by region and month. Although cases of dengue were confirmed for each month of 1974, the highest frequency of positive results was found during the usual epidemic season of May to November. Even then, positive cases rarely represented half of all of the cases tested. As seen in other reports, the epidemic period for the Central region was more prolonged than for the other regions (1).

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The experience with testing encephalitis patients for JEV infection was similar to that with testing DHF patients. In 1974, more patients were submitted from all regions except the North (Table 2). The overall frequency of JEV infections was the same for both years, but the number of undetermined specimens fell substantially.

DISCUSSION: The results of 1974 support the value of the surveillance system. During the 1974 dengue season, the physicians in the provincial hospitals submitted more and better specimens from a greater number of provinces than in 1973. This suggests an increasing awareness of the system on the part of referring physicians and a favorable reaction on their part.

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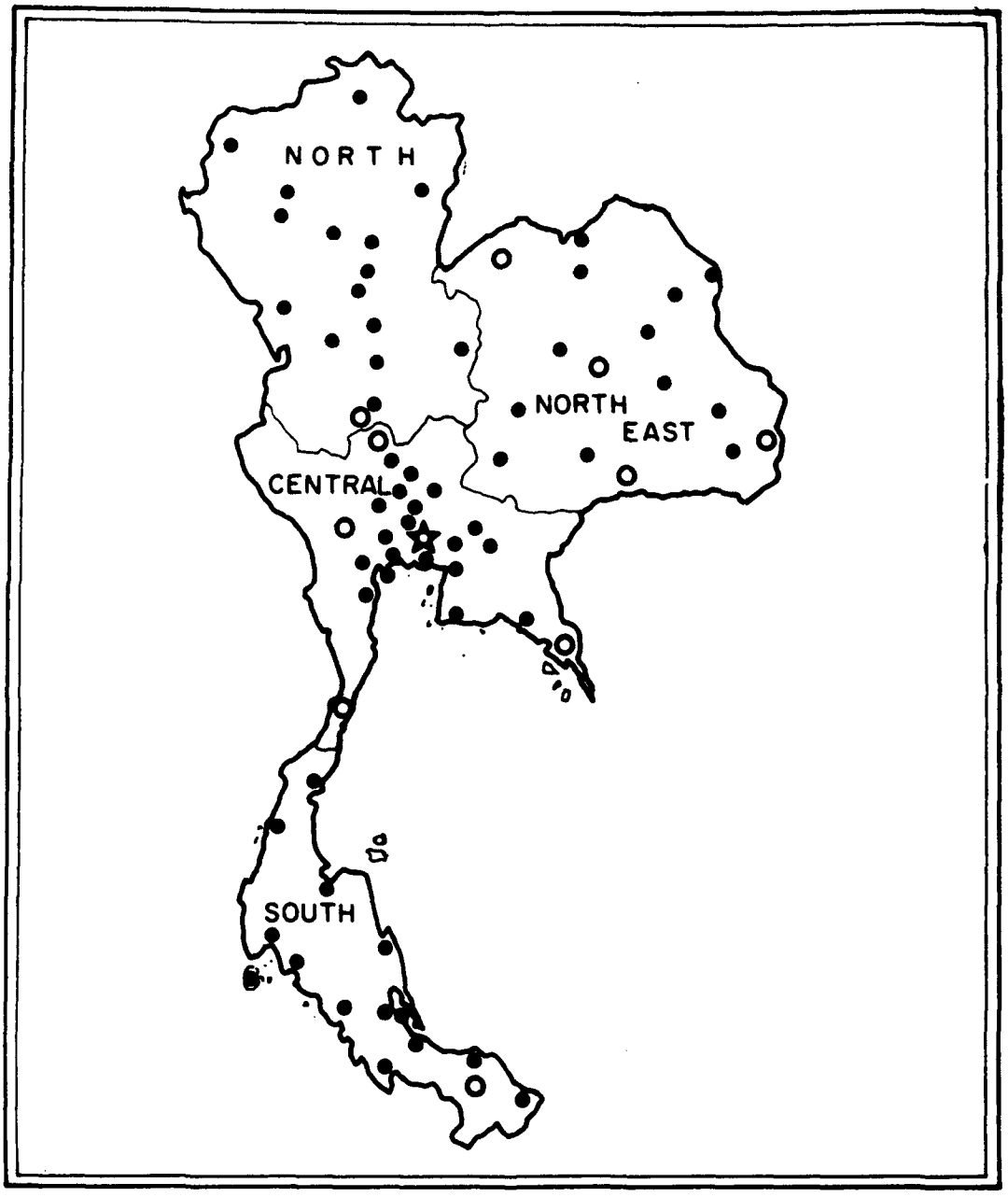

FIGURE I. MAP DEMONSTRATING PROVINCES OR TOWNS OF STUDY (●),(O)

● STUDY AREAS 1973 (60)
● O STUDY AREAS 1974 (60 + 10)

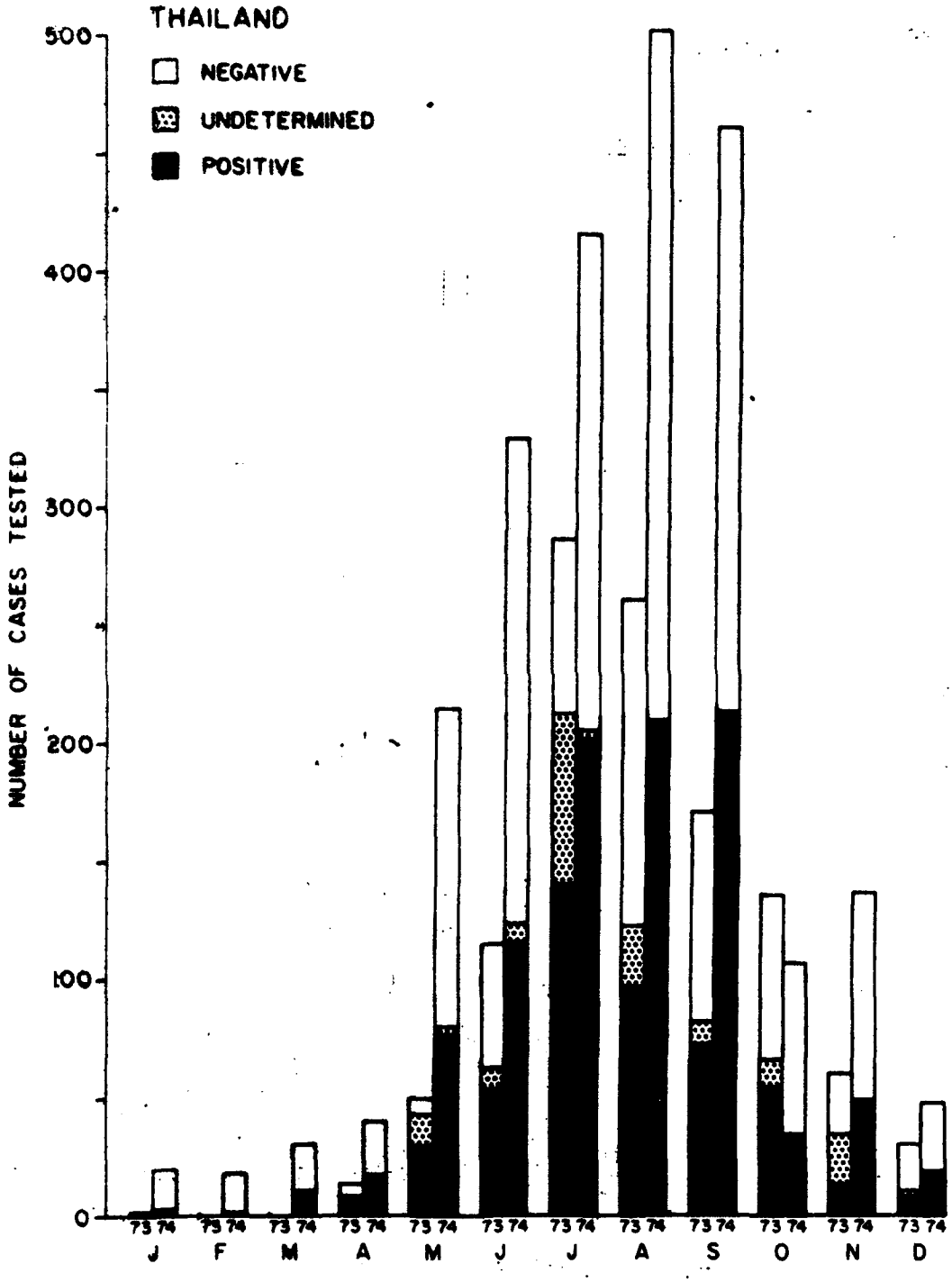

FIGURE 2. CONFIRMATION OF DHF CASES BY HI

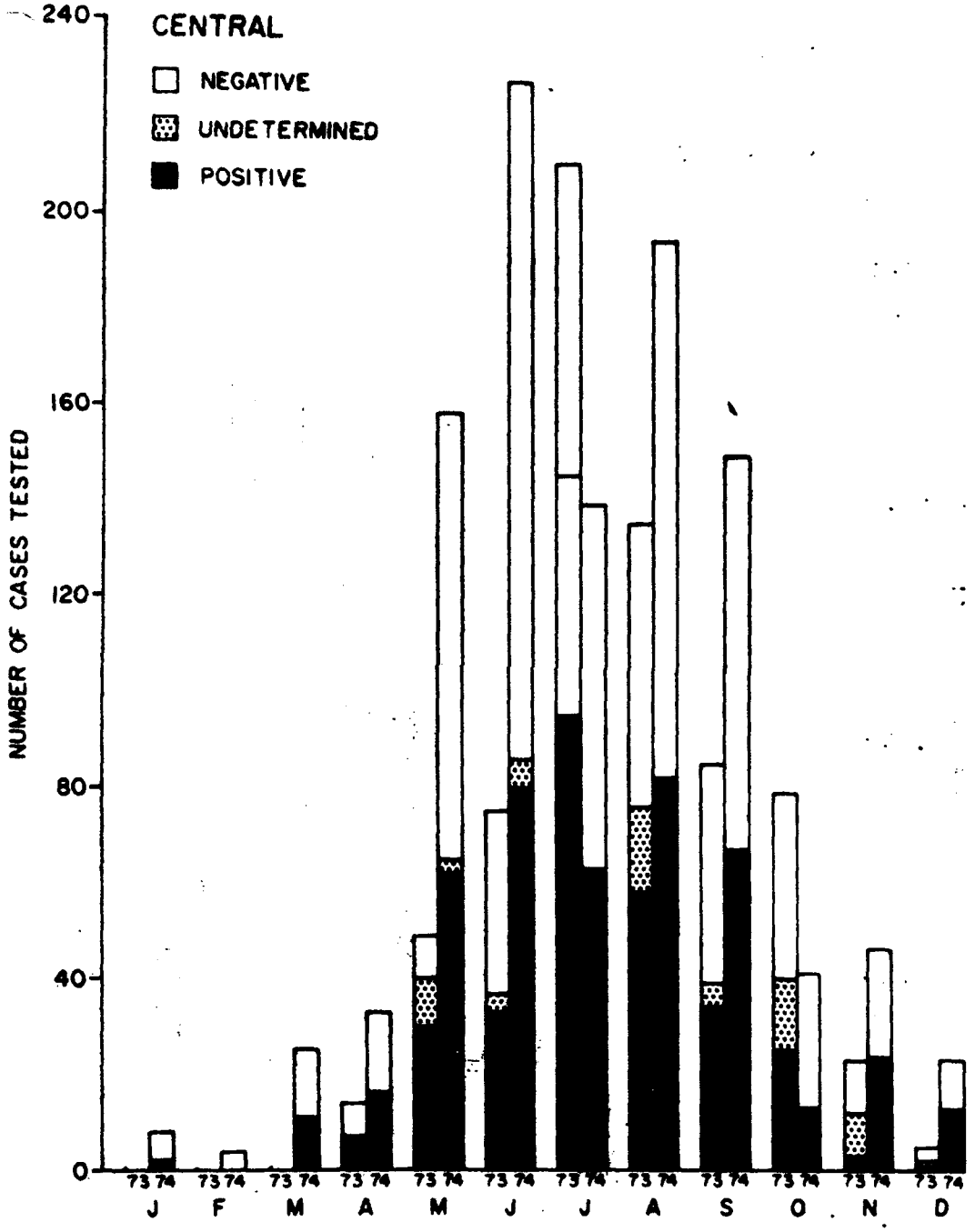

FIGURE 3. CONFIRMATION OF DHF CASES BY HI

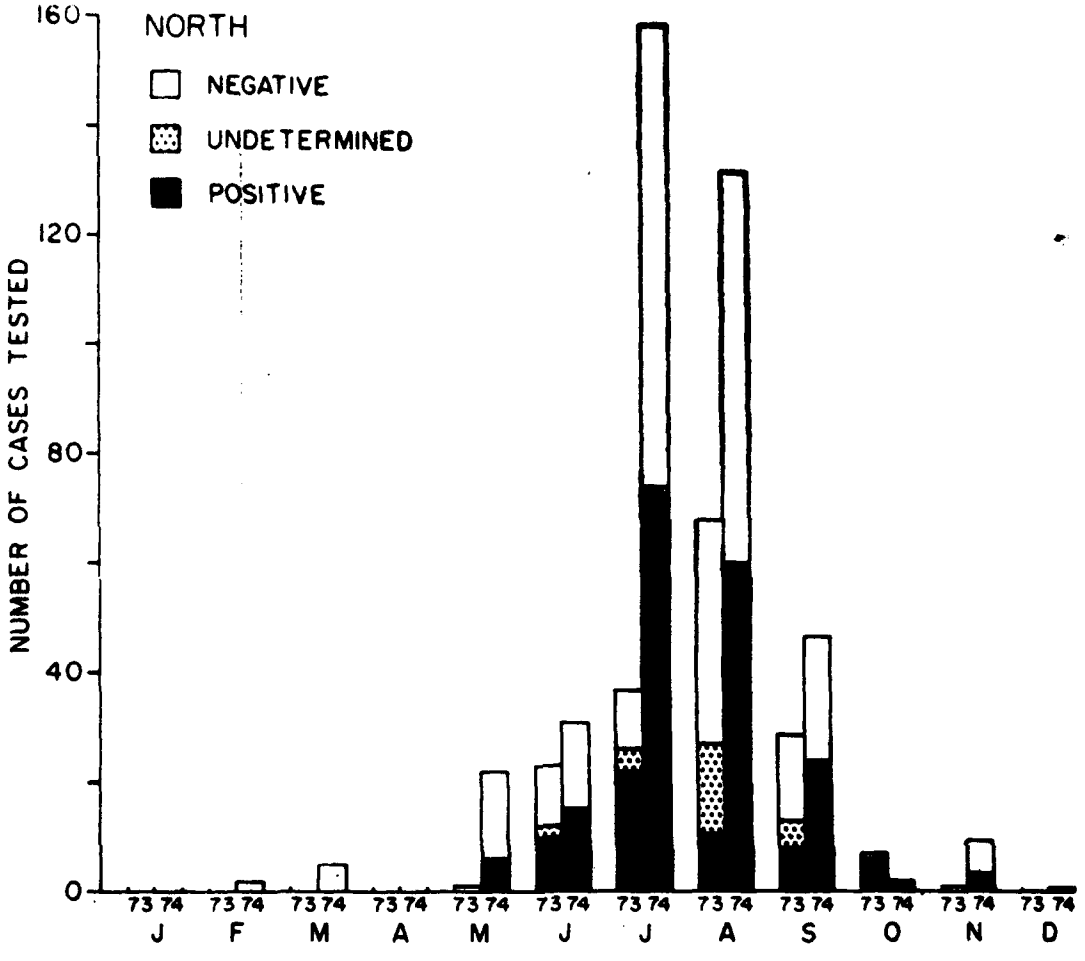

FIGURE 4. CONFIRMATION OF DHF CASES BY HI

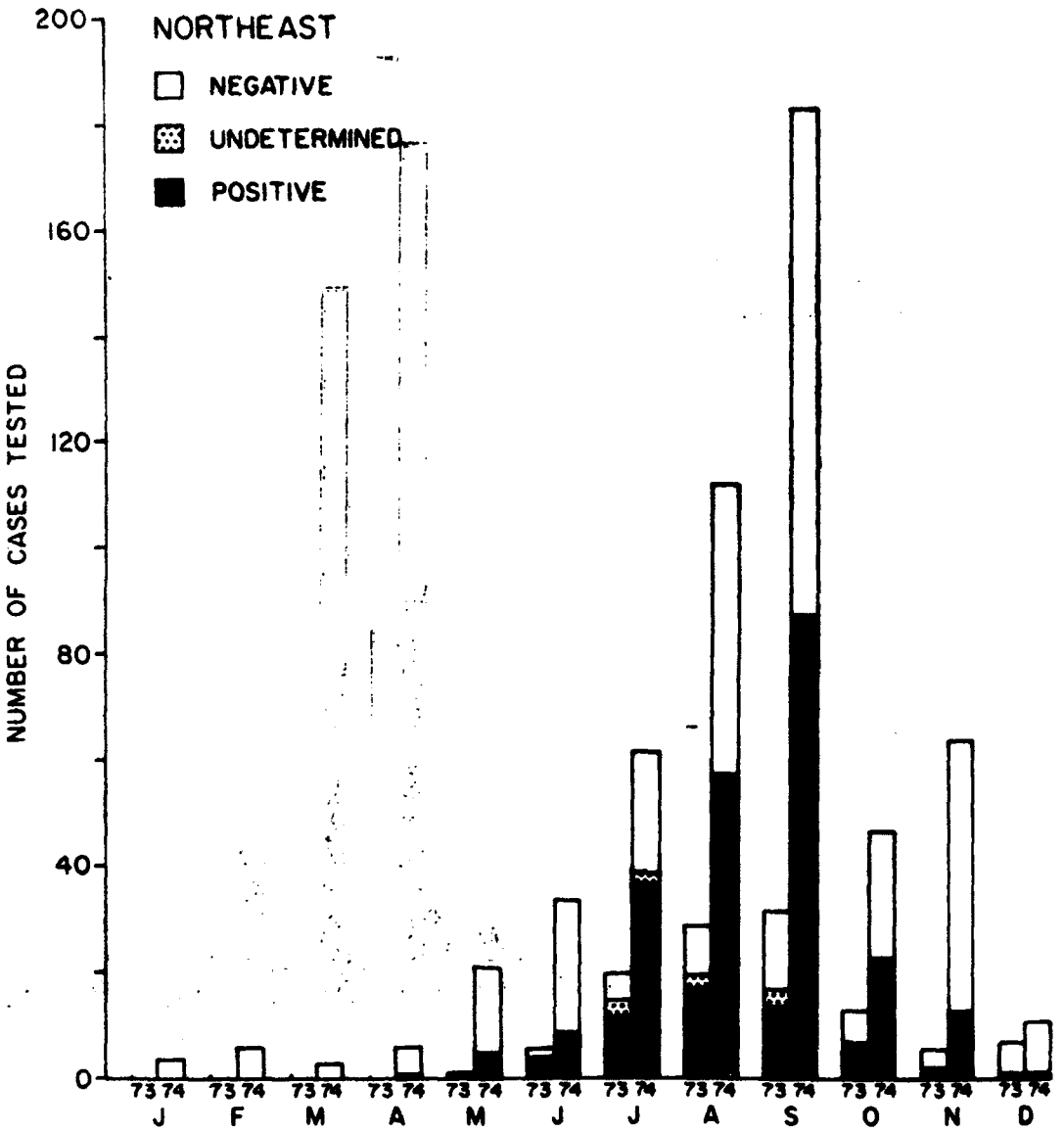

FIGURE 5. CONFIRMATION OF DHF CASES BY HI

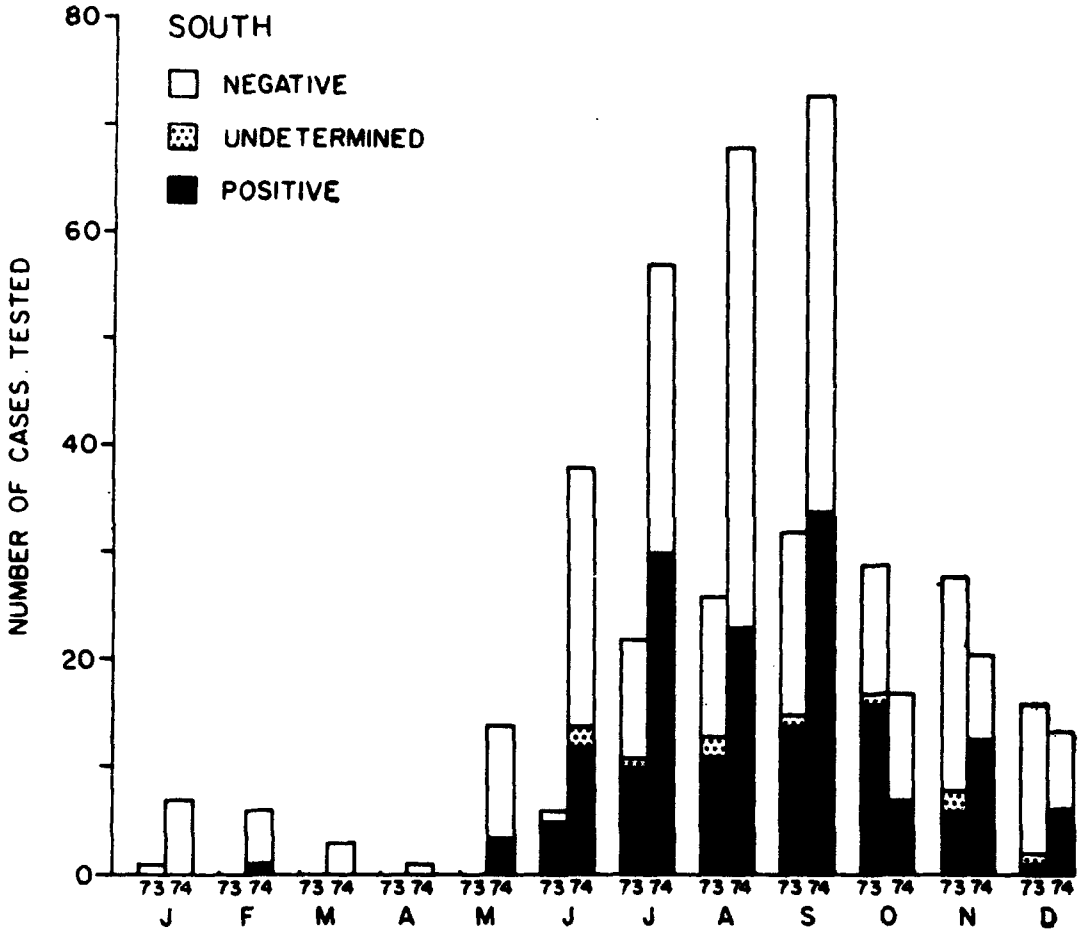

FIGURE 6. CONFIRMATION OF DHF CASES BY HI

Table 1. Results of HI Tests for Dengue Antibody—1973, 1974

Region	No. Patients		Dengue Positive		Undetermined	
	1973	1974	1973 No. (%)	1974 No. (%)	1973 No. (%)	1974 No. (%)
North	249	419	77 (31)	419 (44)	40 (16)	2 (0.5)
Northeast	125	945	62 (50)	945 (26)	9 (7)	3 (0.3)
Central	689	1132	288 (42)	1132 (41)	120 (17)	9 (0.1)
South	173	354	64 (37)	354 (41)	9 (5)	3 (0.8)
Total	1236	2850	491 (40)	2850 (37)	178 (14)	17 (0.6)

Table 2. Results of HI Tests for JEV Antibody—1973, 1974

Region	No. Patients		JEV Positive		Undetermined	
	1973	1974	1973 No. (%)	1974 No. (%)	1973 No. (%)	1974 No. (%)
North	84	18	22 (26)	21 (26)	10 (12)	0 (0)
Northeast	11	79	3 (27)	13 (16)	1 (9)	0 (0)
Central	17	113	5 (29)	32 (28)	0 (0)	2 (1.8)
South	14	26	1 (7)	6 (23)	0 (0)	0 (0)
Total	126	299	31 (25)	72 (24)	11 (9)	2 (0.7)

Dengue Virus Isolation from Human Plasma Inoculated into Mosquitoes

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OBJECTIVE: To compare the results of three techniques of dengue virus isolation from human plasma.

BACKGROUND: An earlier report (1) showed preliminary infection of *Aedes aegypti* mosquitoes with dengue seed virus followed by incubation for 10 days yielded 1 to 3 logs more virus per pool of mosquitoes than was inoculated. Pools of mosquitoes were ground and inoculated into LLC–MK_2 tissue culture for virus isolation and identification by a standard plaque assay. The preliminary results suggested this combined mosquito inoculation/tissue culture (MI/TC) assay was more sensitive than tissue culture alone, but information was needed on the usefulness of this technique for the isolation of dengue virus from human blood.

DESCRIPTION: A collaborative prospective study was done to compare virus isolation results at SEATO Medical Research Laboratory (SMRL) to those at the Pacific Research Section (PRS) of the National Institute of Allergy and Infectious Diseases (NIAID) in Hawaii. Acute plasma samples collected as part of the overall studies of dengue hemorrhagic fever (DHF) at Children's Hospital, Bangkok in 1974 were divided into three identical aliquots and numbered sequentially. Aliquots were frozen promptly at–70°C and not thawed until virus isolation was attempted by: 1) standard plaque assay in LLC–MK_2 tissue culture (TC) cells at SMRL, 2) inoculation of pools of *A. aegypti*, incubation for 10 days, then standard plaque assay in LLC–MK_2 cells at SMRL (MI/TC), and 3) sent to PRS for isolation attempts by inoculation into *A. albopictus* followed by a plaque assay of individual mosquitoes in LLC–MK_2 cells. Virus isolates were identified at SMRL by plaque reduction neutralization tests (PRNT) using type specific hyperimmune mouse ascitic fluid (HMAF). Only the isolates from the standard plaque assay have been completed so far. Identification of isolates at PRS was accomplished by PRNT and complement fixation (CF) using inoculated mosquitoes as the CF antigen.

PROGRESS: There was a striking difference in the number of isolates by the two laboratories. The SMRL TC yielded 11 (14%) isolates from 76 patients compared to 28 (37%) at PRS by MI/TC. Only three SMRL isolates were recovered from plasmas with dengue HI antibody titers of 1:40 or greater (Table 1). On the other hand, fully half of the PRS isolates came from plasmas with HI antibody titers of 1:320 to 1:5120. The results suggest that one reason the MI/TC technique used at PRS recovered more virus isolates was that mosquitoes may be able to disassociate neutralizing antibody from infectious virus particles in the plasma. SMRL did not obtain any isolates by TC that were missed by PRS. There was some difference between the clinical diagnosis of the patients yielding virus isolates to either laboratory (Table 2). Many more isolates were obtained by PRS from patients with DHF grade 2 and 3 than SMRL; patients in these categories generally had higher levels of antibody in the acute plasma samples.

A comparison of the results from MI/TC at SMRL can only be made for 58 plasmas which were carried to completion. Of the 58 plasmas, 6 (10%) were positive by TC, 14 (24%) by MI/TC at SMRL and 18 (31%) by MI/TC at PRS (Table 3). Five plasmas yielded isolates by the SMRL MI/TC only; their identity is still in doubt. One plasma was positive by SMRL TC and at PRS but negative by MI/TC at SMRL. On the other hand, four plasmas yielded isolates by both mosquito inoculation techniques. Some of the differences in results are probably due to technical problems at SMRL that still need to be solved.

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DISCUSSION AND SUMMARY: A collaborative study for the comparison of three techniques for dengue virus isolation was carried out with PRS/NIAID. Techniques using mosquitoes as amplifying hosts for dengue replication prior to isolation of virus in LLC–MK_2 cells yielded a greater number of isolates than a standard tissue culture plaque assay method. The greatest improvement in isolation results was found in plasmas containing dengue HI antibody at titers of 1:40 or higher. It is presumed that inoculation of plasma into mosquitoes permits dissociation of neutralizing antibody from some infectious virus particles. The benefit of mosquito inoculation is two–fold: 1) the mosquito may strip off interfering antibody; 2) the mosquito allows small amounts of virus to multiply to levels that can be more easily detected in a LLC–MK_2 tissue culture system. The mosquito inoculation step will be included in future attempts at dengue virus isolation.

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Table 1. Dengue HI Antibody Titers of Plasma Specimens Yielding Virus Isolates

Dengue Antibody Titer [a]	No. Tested	No. of Virus Isolates	
		SMRL [b] LLC–MK_2	PRS [c] MI/LLC–MK_2
<20	7	6	6
20	2	2	2
40	2		1
80	3		2
160	3	2	3
320	7		6
640	11		2
1280	7		1
2560	15		3
5120	11	1	2
10240	5		
≥20480	3		
TOTAL	76	11	28

a Reciprocal plasma dilution

b Virus isolates made at SEATO Medical Research Laboratory by direct inoculation of LLC–MK_2 tissue culture

c Virus isolates made at the Pacific Research Section by inoculation of mosquitoes before attempting virus isolation in LLC–MK_2 tissue culture

Table 2. Diagnosis of Patients Yielding Virus Isolates

Diagnosis	No. Patients	No. of Virus Isolates	
		SMRL [a] LLC–MK_2	PRS [b] Mosquito/LLC–MK_2
Dengue Fever	5	2	3
DHF grade 1	6	2	3
grade 2	31	4	11
grade 3	23	1	7
grade 4	10	2	4
unspecified	1	0	0
Total dengue	76	11	28
Non–dengue	25	0	0

a Virus isolates made at SEATO Medical Research Laboratory by direct inoculation of LLC–MK_2 tissue culture

b Virus isolates made at the Pacific Research Section by inoculation of mosquitoes before attempting virus isolation in LLC–MK_2 tissue culture

Table 3. Relative Number of Dengue Virus Isolates Obtained from 58 Human Plasmas by Three Techniques

PRS MI/TC[a]	SMRL MI/TC	SMRL TC [b]	No. Dengue Isolates
+	0	+	1
+	+	+	5
+	+	0	4
+	0	0	8
0	+	0	0[c]
18	9	6	18

+ means test positive; 0 means test negative

a Mosquito inoculation followed by tissue culture isolation technique

b Tissue culture only

c Five isolates obtained only by SMRL MI/TC have not yet been identified

Rapid Detection of Dengue Virus Antigen and Antibody by Counterimmunoelectrophoresis (CEP)

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OBJECTIVE: To determine the ability of a CEP test to detect dengue antigen and antibody in human serum.

BACKGROUND: Because the manifestations of dengue hemorrhagic fever (DHF) develop very rapidly, a quick screening test for the detection of dengue antigen and antibody in patient serum would be beneficial to physicians. The use of CEP for making a diagnosis of other viral infections led to an attempt to apply the technique to dengue infections.

DESCRIPTION: A collaborative study was conducted in which sera from selected DHF patients in Children's Hospital collected and tested by virus isolation and hemagglutination inhibition tests in the Dept of Virology, SEATO Medical Research Laboratory (SMRL) were tested under code by CEP in the Dept of Pathobiology, Mahidol University. The CEP technique has been described (1). Antibody was detected using 20% suspensions of suckling mouse brain prepared at SMRL representing dengue types 1 to 4 and Japanese encephalitis virus. Dengue antigen was detected by screening with hyperimmune mouse ascitic fluid (dengue 1 and 4) and rabbit antisera (dengue 2 and 3). Few sera were tested with the anti-dengue 3 sera.

PROGRESS: Two to four sera were tested from 10 different patients with dengue infections documented by virus isolation (five cases), a four-fold rise in antibody (four cases) or a high fixed antibody titer (one case). In every patient, the first serum specimen gave a positive reaction for antibody by CEP with at least one dengue antigen, whether or not virus was isolated or antibody was detected by hemagglutination inhibition. On the other hand, antigen was detected by a reaction with dengue antibody in only three acute specimens; dengue virus was isolated from all three (Table 1). The results suggest CEP may be able to detect dengue antigen in some viremic patients but may not be specific enough for the detection of dengue antibody.

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Table 1. Detection of Dengue Antigen in Viremic Human Serum

Patient No.	Virus Isolate	CEP Reaction with Type of Antibody			
		Dengue–1	Dengue–2	Dengue–3	Dengue–4
73–9	D1	0	0	ND	0
73–17	D3	0	0	+	0
73–19	D3	0	0	ND	0
73–68	D2	0	0	ND	+
73–41	D2	+	+	ND	0

D = Dengue
ND = Not done

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The Pathogenesis of Dengue Hemorrhagic Fever. The Role of Biological Mediators: Histamine and Serotonin

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OBJECTIVE: A study of biological mediators of shock in dengue hemorrhagic fever.

BACKGROUND: Dengue hemorrhagic fever (DHF) differs from "normal" or "unmodified" dengue in the development of hemorrhagic phenomenon (a positive tourniquet test), "hypotension", (a low blood pressure and/or a narrowed pulse pressure), a decrease in plasma volume, (a rising hematocrit) and thrombocytopenia (a rapid drop in platelet count). The differences appear to be related to the formation of antigen antibody complexes (1), and it has been suggested that this is related to an individual experiencing a second dengue infection (2). These changes have a rapid onset suggesting the involvement of short-lived biochemical mediators.

At least two phenomena have been observed during the development of this illness which may be the source of these mediators. Observations suggest that there is activation of the complement system by antigen-antibody complexes with liberation of pharmacologically active components C3A and C5A (3). These low molecular weight polypeptides are potently vasoactive and their release leads to a marked increase in vascular permeability (4). They may act directly on the vasculature or by liberating histamine, slow reacting substance (SRS-A) and/or heparin from mast cells and white blood cells.

The other phenomenon is the decrease in platelets (5). Platelet counts often fall in a matter of hours from a normal level of approximately 250,000/mm^3 to as low as 11,000/mm^3. Platelets also return rapidly to supra-normal levels during the recovery phase (6). As the half life of the platelet is only two to three days this suggests that there is acute lysis of many platelets with supra-normal replacement from a hyperactive bone marrow. A similar but less marked phenomenon has also been noted with white blood cells (5).

The reason for the acute decrease in platelets is not clearly understood and requires further study. The lysis of platelets may lead to the sudden release of several potent vasoactive agents. Histamine, serotonin and heparin are all found in platelets and could be released into the circulation.

C3A and C5A are labile polypeptides and can not yet be accurately measured (7). SRS-A which is released from mast cells, has not been characterized and can only be measured inaccurately by a biological assay (8). Heparin is stored in both platelets and mast cells; it also is difficult to measure in serum.

Serotonin is manufactured in fairly large amounts in the chromaffin cells of the gastrointestinal tract. This vasoactive amine has a rapid turnover time. Most of the serotonin manufactured appears as metabolites in the urine within 24 hours; however, a small amount of it is taken up and stored in platelets (9). In man no serotonin is found in mast cells (10). The major metabolite of serotonin, 5 Hydroxy-indolacetic/acid (5 HIAA), can be measured in the urine by colorometric analysis.

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Histamine is a major storage product of mast cells with a small amount being stored in platelets (9). In the past it could be measured only by biological assay but recently a sensitive radioenzymic assay has been developed (11). The purpose of this study was to determine whether the excretion of histamine or 5 HIAA increased in the urine during the development or the course of DHF as compared to other febrile diseases and normal controls.

DESCRIPTION: This study was an integral part of other dengue studies performed in collaboration with the Children's Hospital of Bangkok during 1974. During the dengue epidemic season from 24 June to 26 August 1974 all patients admitted to the ward service with an admission diagnosis of DHF and appropriate febrile and afebrile controls were accepted for study. Blood samples were taken daily in heparinized syringes for the first five days in hospital and then approximately 15 and 30 days following hospitalization. Plasma was submitted for virus isolation using a direct and delayed plaque technique previously described and a mosquito isolation technique (see elsewhere this report). Plasma was also used for the detection of antibodies to dengue types 1–4 and Japanese Encephalitis virus using a hemagglutination inhibition (HI) technique. Urines, collected over either 8 or 12 hour periods, were obtained from each patient in plastic bottles and stored on wet ice. At the conclusion of the collection period these were divided into aliquots in plastic containers using toluene as a preservative and frozen at −70°C. A white blood count, differential count, platelet count and hematocrit were performed on each blood obtained. The clinical status of each patient was assessed by a physician at least once every 12 hours and usually more often. All clinical and laboratory details were recorded on a flow sheet, which was kept on each case.

Patients to be further studied for the presence of biological mediators were selected retrospectively after all clinical and laboratory information was evaluated.

PROGRESS: During this two month period at the height of the dengue season only 64 patients with an admission diagnosis of DHF were collected. This, however, represented almost 50% of the dengue patients collected through the entire year, as there was a low incidence of disease during 1974. Of these 64 patients, 11 or 18% did not have laboratory evidence of dengue virus infection, one patient was not adequately followed and three patients died. From the three patients who died no convalescent sera was obtained and therefore no judgement could be made on the patient's prior experience with dengue. Table 1 shows a breakdown on the clinical grading and type of convalescent antibody response seen in the 49 patients who were studied.

Of these patients five were selected who exhibited DHF grades 1 or 2 and five were selected who exhibited DHF grades 3 or 4. Three patients with bacterial infections and five non–infected children were selected as controls. All urine samples collect on these children from the time of admission until two days after the period of shock were submitted for biochemical analysis of creatinine, 5 HIAA and histamine. The 5 HIAA and creatinine assays were done at SEATO Medical Research Laboratory. Urine for histamine analysis was forwarded through Walter Reed Army Institute of Research to Dr. Michael Beaven at the NIH in Bethesda, Maryland to be tested for histamine by radioenzymic assay. When all of the assays are completed the data will be examined to determine whether the excretion of histamine or serotonin breakdown products was related to the development or severity of DHF.

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Table 1. Clinical Grading and Type of Convalescent HI Antibody Responses seen in 49 Dengue Patients Collected Between June 24 and August 26, 1974

Grade of Disease	Primary Infection $\leq$ 1:640	Secondary Infection $>$ 1:640
UF*	1	3
1 & 2	5	22
3	2	12
4		4
TOTAL	8	41

* Undifferentiated fever

Radioimmune Assay Inhibition Tests for the Detection of Antibody to Hepatitis B Surface Antigen

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OBJECTIVE: To compare the radioimmune assay inhibition (RIAI) test for antibody to hepatitis B surface antigen (anti–HB_s) using two radioimmune assay (RIA) techniques.

BACKGROUND: The RIAI for anti–HB_s was developed in this laboratory using the Ausria I RIA kit manufactured and sold by Abbott Laboratories (SEATO Medical Research Laboratory Annual Report 1973–1974). To the procedure for the Ausria I, an initial absorption step was added. The sera, whose antibody content was to be determined, was used to absorb a standard amount of antigen. A change in the technical aspect of this test was brought about by the discontinuation of production of the Ausria I kit and its replacement by the manufacturer with a newly developed Ausria II kit. This change in material necessitated a series of comparative tests to insure that the results of the RIAI based upon the Ausria II ($RIAI_{II}$) were comparable to those based upon the Ausria I ($RIAI_I$).

DESCRIPTION: In February 1975 the Ausria I kit on which the $RIAI_I$ was based was withdrawn from the market. A final order of Ausria I kits and an equal number of the replacement Ausria II kits were provided by the manufacturer for cross testing and standardization. These kits were used to compare both the RIA and the RIAI tests in our laboratory.

The major technical difference between the Ausria I and the Ausria II was a change in the antibody carrier from a polystyrene tube to a polystyrene bead. The technique for the $RIAI_I$ was presented in last year's annual report. The initial absorption technique for the Ausria II is briefly reported: the RIAI was performed using a standard dilution of serum containing a known amount of antigen. Exactly 0.1 ml of this antigen dilution was incubated with 0.1 ml of each serum to be tested. After 1½ hours incubation a polystyrene bead was introduced into the well and submerged in the mixture. From this point the test was performed using the directions for the Ausria II kit provided by the manufacturer and is essentially the same as with the Ausria I.

Included in each test run were seven negative controls, testing a pool of human serum shown to have neither HB_sAg or anti–HB_s activity. The standard antigen dilution which was used as a maximum for the RIAI, was also tested in seven replicates. The number of counts per minute (CPM) in each test was determined using a gamma ray spectrometer.

The percent radioimmune assay inhibition (% RIAI) was calculated using the following formula:

$$\frac{D - X}{D} \times 100 = \% \text{ RIAI}$$

D is the mean of the CPM of the standard antigen dilution from which the mean of the negative controls had been subtracted and X is the CMP of the serum–antigen mixture following a similar manipulation.

Data from the $RIAI_I$ and $RIAI_{II}$ were compared on the basis of the percent RIA inhibition. Also, a positive or negative score was assigned to each test using 50% inhibition as a cut–off point for differentiating positives from negatives.

PROGRESS: In order to establish the appropriate dilution of antigen to use in the RIAI tests, antigen extinction curves using a sera containing HB_sAg/adw were run using both RIA tests. The results were found to be almost identical (Figure 1). Dilutions of 1:400 and 1:800 were selected as candidates for the standardized antigen for use in the RIAI tests. These dilutions were selected because they showed 50% or less of the CPM of the highest counting antigen dilution and were located on the steepest part of the antigen dilution curve. In this part of the curve, small changes in the concentration of antigen in the substance tested should lead to large changes in the amount of I^{125}-labelled antibody complexed to the antigen.

Dilutions of serum known to contain a high titer of anti-HB_s (Serum PT) were tested by both tests using dilutions of HB_sAg/adw of 1:400 and 1:800. Figure 2 illustrates the percent RIAI of the dilutions of antibody using a 1:800 dilution of the HB_sAg/adw antigen. Use of the 1:400 dilution of the HB_sAg/adw antigen produced similar curves with both tests but the RIA was inhibited by lower antibody dilutions and therefore was less sensitive. For this reason a 1:800 dilution of HB_sAg/adw was chosen as the standard antigen dilution for the RIAI with both the Ausria I and the Ausria II ktis.

A panel of 100 sera from a Thai population known to have a high prevalence of anti-HB_s was tested by RIAI using both techniques and the standard 1:800 dilution of HB_sAg/adw. Figure 3 illustrates the relationship between the percent RIAI on these 100 sera using the $RIAI_I$, versus the $RIAI_{II}$. The correlation coefficient (r) of results obtained from these two techniques was 0.96. This very high correlation indicates that the two tests are measuring the same variable. The proportion of common variance (r^2) was 0.93, indicating that 93% of the variation in one test is accounted for by the variation of the other test.

When the scores assigned to each test on the basis of the 50% cut-off point were examined, 45 of the 100 sera were positive by the $RIAI_{II}$ and 38 of the 100 were positive by the $RIAI_I$ (Table 1). The correlation coefficient (r_{phi}) of the tests scored in this way, was only slightly lower than that derived from the numerical data, again indicating the similarity of the two tests (Table 2).

The 100 sera were also tested by the passive hemagglutination test (PHA, Electronucleonics Inc.) and the immunoelectroosmophoresis test (IEOP) (Table 1). The correlation coefficients (r_{phi}) were also calculated between these tests and both of the RIAI tests (Table 2). The low correlation coefficients between the IEOP and other tests is indicative of the lack of sensitivity of the former as has been shown previously. The PHA identified anti-HB_s in 34 of 100 sera tested. All of these 34 sera were also positive by $RIAI_{II}$.

Table 1. A Comparison of Four Tests for Anti-HB_s Results of 100 Sera

Test	Pattern of Positive Results					Sera positive for each test
RIAI						
AUSRIA II	X	X	X	X	X	45
AUSRIA I	X	X	X			38
PHA	X	X		X		34
IEOP	X					11
Total sera positive by tests indicated	11	20	7	3	4	

Table 2. Correlation Coefficient r_{phi}

AUSRIA II	vs	AUSRIA I	0.87
AUSRIA II	vs	PHA	0.79
AUSRIA I	vs	PHA	0.78
PHA	vs	IEOP	0.49
AUSRIA I	vs	IEOP	0.46
AUSRIA II	vs	IEOP	0.41

$$\text{Where } r_{phi} = \frac{BC - AD}{\sqrt{(A + B)(C + D)(A + C)(B + D)}}$$

In order to increase the confidence in the $RIAI_{II}$ an additional 100 sera, taken from the same population, were added to the original panel (Table 3). In this experiment the $RIAI_{II}$ identified anti-HB_s in 83 (41%) of the 200 sera as compared to 69 (35%) identified by the PHA. The differences seen here were due to 15 sera that were positive only by $RIAI_{II}$ and one serum which was positive only by PHA. The correlation coefficient (r_{phi}) was larger than that found with the original panel of 100 sera indicating an increased between-test reliability.

Table 3. A Comparison of Two Tests for Anti—HB_s Results of 200 Sera

Test	Pattern of Positive Results			Sera positive for each test
RIAI				
AUSRIA II	X	X		83
PHA	X		X	69
Total sera positive by tests indicated	68	15	1	

DISCUSSION AND SUMMARY: The $RIAI_{II}$ has been used by this laboratory for the past year to identify anti—HB_s in various populations. This test was shown to be slightly less sensitive than the PHA. The introduction of the Ausria II test with the withdrawal of the Ausria I test from the market required the series of comparative tests reported here. The $RIAI_{II}$ proved to have an increased sensitivity over the $RIAI_{I}$. Furthermore, using this test instead of the $RIAI_{II}$ there appeared to be fewer sera in which anti—HB_s was identified by PHA and not by RIAI. Proving increased sensitivity of the $RIAI_{II}$ remains a problem, as many of these positives cannot be confirmed by PHA. Nonetheless, the $RIAI_{II}$ has now replaced the $RIAI_{I}$ in this laboratory for the initial identification of sera containing anti—HB_s.

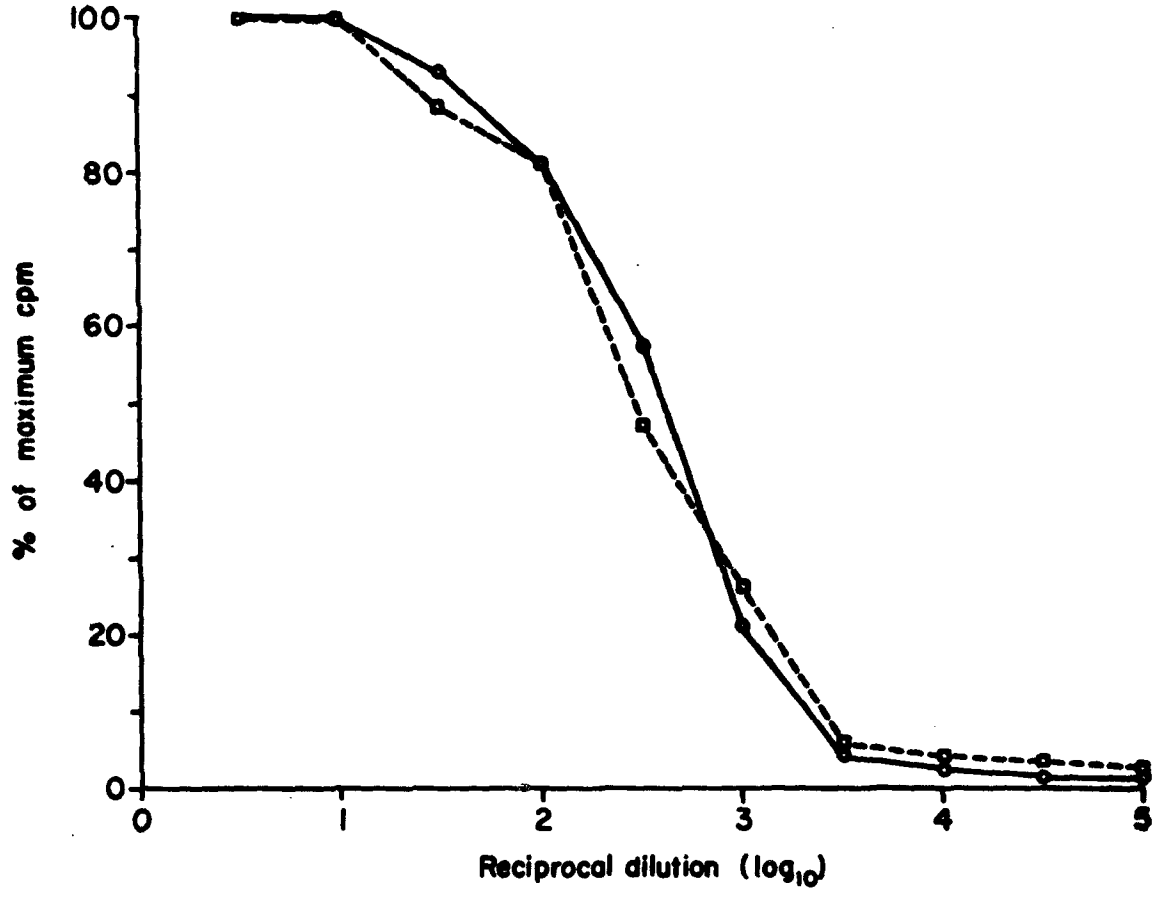

Figure 1. Antigen dilution extinction curves of sera containing HB_sAg/adw (CF - titer 1:256) tested by radioimmune assays using both the Ausria I and the Ausria II tests provided by Abbott Laboratories.

▫----▫ Ausria I, o——o Ausria II

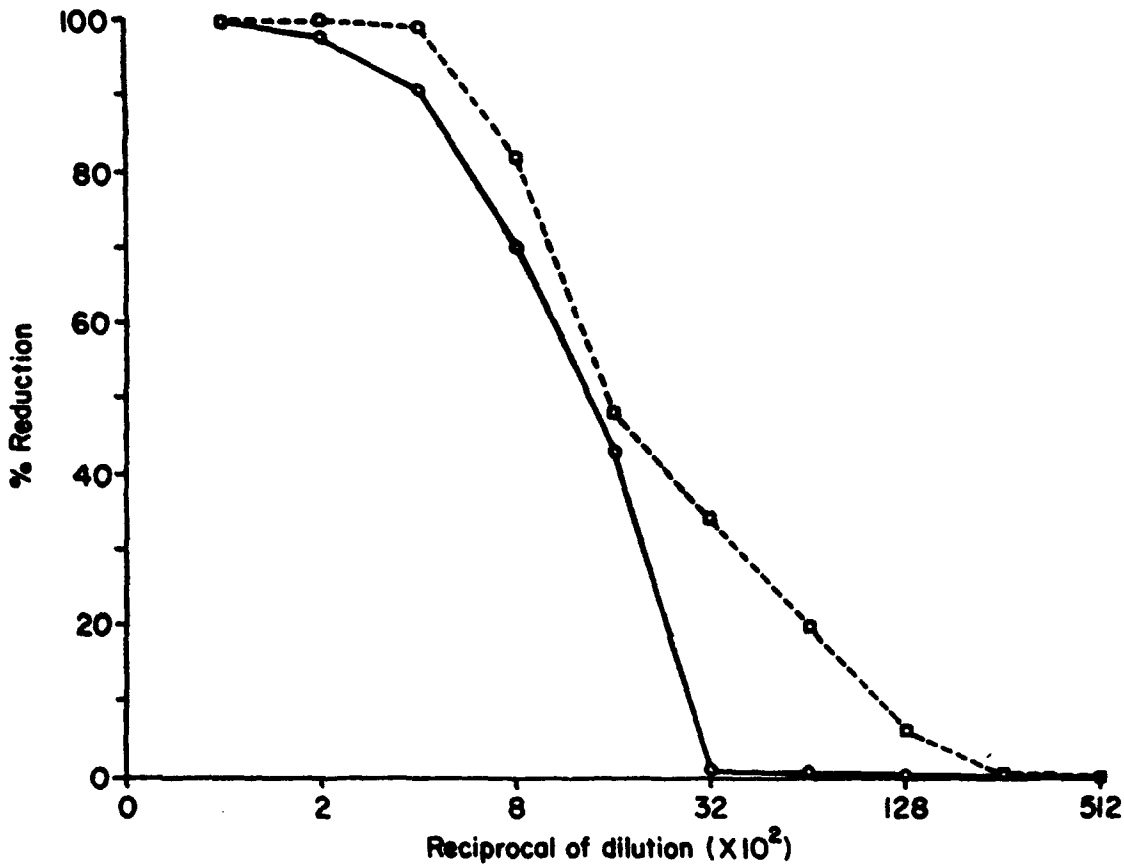

Figure 2. Radioimmune assay inhibition curves on dilutions of a serum containing a high titer of anti-HB_s (PT, CF titer = 1:64). The Ausria I and Ausria II kits were used with a standard antigen dilution of HB_sAg/adw of 1:800.

Ausria I, Ausria II

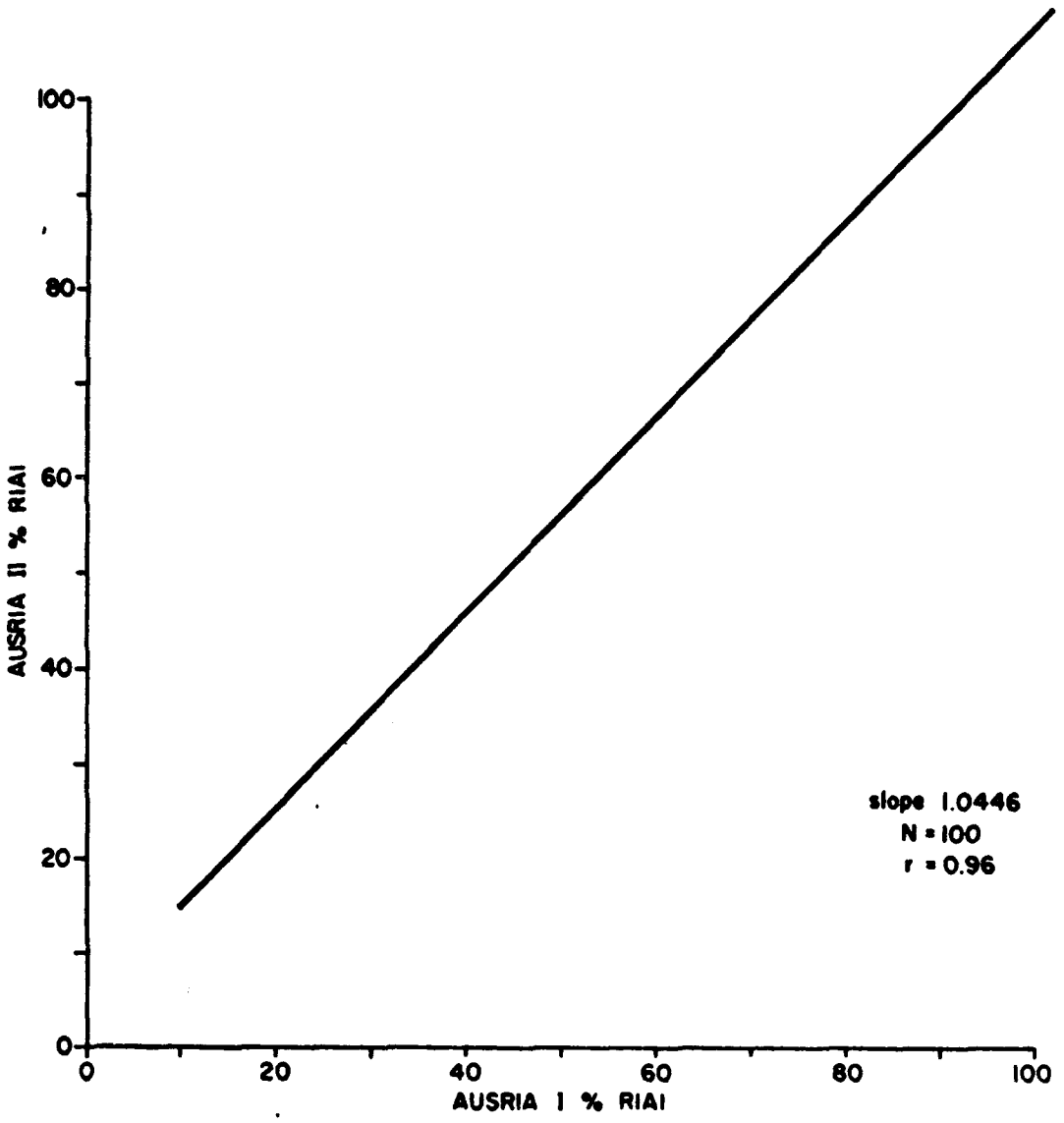

Figure 3. Relationship between the percent radioimmune assay inhibition as shown by the $RIAI_I$ and the $RIAI_{II}$

Anti–Hepatitis B Serum Production in Laboratory Animals

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OBJECTIVE: To produce antisera to Hepatitis B surface antigen (HB_sAg) for use in the determination of antigen subtypes.

BACKGROUND: This is a continuation of work which was previously reported (1). The detection of subtypes of Hepatitis B surface antigen requires antisera containing specific antibodies to each subtype determinant. Previously these antisera were prepared using rabbits, who were exsanguinated six weeks after the initiation of the immunization procedure (2). This method of preparation produced antisera with a high titer against homologous antigen; however, because of impurities in the original immunogen, the antisera were often contamintated with anti–human serum protein activity. This anti–human serum activity interfered with immunodiffusion (ID) tests (3) and required absorption with normal human sera. The present study was designed to determine if subtype specific antisera free of anti–human serum activity could be produced in rabbits by selecting the time of bleeding.

DESCRIPTION: Rabbits of 2.5 to 4.0 Kg bodyweight were used. Five milliliters of blood were taken from the peripheral ear vein of each rabbit before incubation and nearly every week post inoculation. Anti–HB_s activity as well as anti–human serum protein activity were tested by immunoelectroosmophoresis (IEOP) and titered by complement fixation test (CF). A cesium chloride purified fraction of HB_sAg/adr (EH–17) prepared by Electronucleonics, Inc., Bethesda, Maryland was emulsified with an equal volume of Freund's complete adjuvant (2). Four rabbits free from anti–HB_s activity were inoculated with 0.25 ml of antigen–adjuvant emulsion, intradermally, into each of four sites on the thighs and back. An identical dose of the same antigen was given four weeks later. Anti–HB_s and anti–human serum protein activities were studied once a week from two to ten weeks.

All blood was tested for anti–HB_s and anti–human serum protein activities by IEOP and CF. The titer of these antibodies was determined by CF and the specificity of the antisera was identified by ID.

PROGRESS: Four rabbits were immunized with HB_sAg/adr (EH–17). Of these four, one died of unknown cause four weeks after immunization.

In the remaining three, antibodies to HB_sAg/adr with CF titers of 1:2 to 1:16 had appeared by the first bleed, two weeks after immunization (Table 1 and Figure 1). CF titers of antibodies increased slowly reaching 1:8 to 1:32 by the fourth week just prior to receiving the booster dose of immunizing antigen. Antisera reached its maximum titer of 1:64 to 1:128 by five weeks, one week after the booster dose. After the fifth week the titer remained stable until the rabbits were exsanguinated at 8–9 weeks.

The specificity of individual rabbit sera were determined by the ID test. Two rabbit sera (R26 and R28) formed definite precipitin lines with reference antigens, but only specific d spurs were observed (Table 1 and Figure 2) suggesting they contained only ad subtype specific determinant antibodies. Another rabbit serum (R29) gave specific reactions with the reference antigens, with both d and r spurs, suggesting it contained subtype specific a, d and r antibodies.

Antibody to normal human serum protein was observed only transiently and at low titer (Table 1 and Figure 1). The interference of anti–human serum protein disappeared in the ID test at 7 to 8 weeks post–inoculation. Anticomplementary activity of all three rabbit antisera was minimal and did not interfere with the interpretation of the tests.

DISCUSSION: The method of immunization with HB_sAg/adr in rabbits proved to be satisfactory for the production of subtype specific antisera in one (R 29) out of three rabbits. In the other two, (R 27 and R 28) a strong d spur was produced but no easily discernible reaction with the r antigen was observed. In R 29, anti—human serum activity in ID tests disappeared by the seventh week. This allowed the use of antiserum from this rabbit in the ID test without preabsorption with normal human serum.

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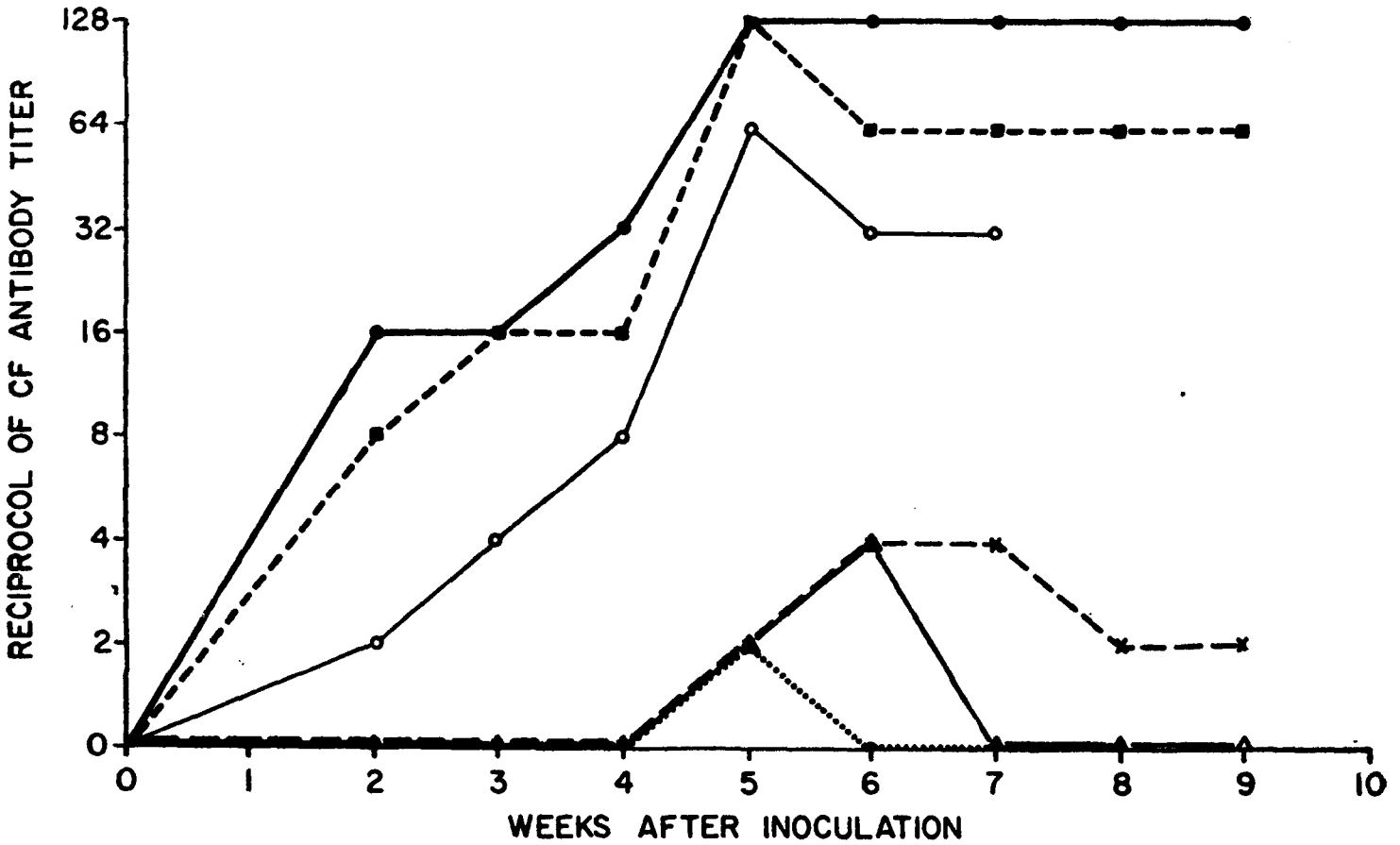

FIGURE 1 Anti-HB_S and anti-human protein responses in rabbits immunized with purified HB_SAg/adr (EH-017).

- Anti-HB_S R26
- Anti-HB_S R28
- Anti-HB_S R29
- Anti-human protein R26
- Anti-human protein R28
- Anti-human protein R29

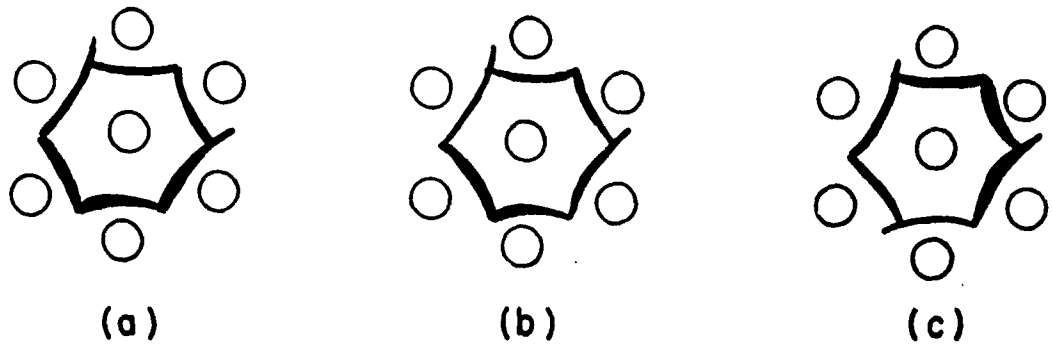

Figure 2. Patterns of immunodiffusion reactions observed with reference HB_sAg. In each pattern, reference HB_sAg/ayw were placed in the top and right upper wells, HB_sAg/adr in right lower and bottom wells and HB_sAg/adw in left upper and left lower wells. The central wells contain: (a) R. 26 anti-adr, (b) R. 28 anti-adr, and (c) R. 29 anti-adr rabbit sera.

Table 1. Production of Anti-HB_sAg/adr in Rabbits

Rabbit No.	Time After Immunization	Anti-HB_s				Anti normal human protein		
		Immunodiffusion		IEOP	CF Titer	Immunodiffusion	IEOP	CF Titer
		d Spur	r Spur					
R 26	Pre-immunization	ND*	ND	–	ND	ND	–	ND
	2 weeks	–	–	+	1:2	–		<1:2
	3 weeks	ND	ND	+	1:4	ND		<1:2
	4 weeks	ND	ND	+	1:8	ND		<1:2
	5 weeks	+	–	+	1:64	+		1:2
	6 weeks	+	–	+	1:32	+		<1:2
	7 weeks	+	–	+	1:32	+		<1:2
R 28	Pre-immunization	ND	ND	–	ND	ND	–	
	2 weeks	–	–	+	1:8	–		<1:2
	3 weeks	ND	ND	+	1:16	ND		<1:2
	4 weeks	ND	ND	+	1:16	ND		<1:2
	5 weeks	+	–	+	1:128	+		1:2
	6 weeks	+	–	+	1:64	+		1:4
	7 weeks	+	–	+	1:64	+		1:4
	8 weeks	+	–	+	1:64	–		1:2
	9 weeks	+	–	+	1:64	–		1:2
R 29	Pre-immunization			–	ND	ND	–	ND
	2 weeks	–	–	+	1:16	–		<1:2
	3 weeks	ND	ND	+	1:16	ND		<1:2
	4 weeks	ND	ND	+	1:32	ND		<1:2
	5 weeks	+	+	+	1:128	+		1:2
	6 weeks	+	+	+	1:128	+		1:4
	7 weeks	+	+	+	1:128	–		<1:2
	8 weeks	+	+	+	1:128	–		<1:2
	9 weeks	+	+	+	1:128	–		<1:2

Note: ND* = Not done

Hepatitis B Virus Infections in Americans in Southeast Asia

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OBJECTIVE: To determine the epidemiology of hepatitis in American military personnel exposed to populations with endemic hepatitis and a high prevalence of HB_sAg carriers.

BACKGROUND: Until recently only historical evidence was available to document infection with agents causing viral hepatitis. In the past 10 years, however, investigations of hepatitis B, initially stimulated by the discovery of hepatitis B surface antigen, have provided serological evidence of infections with hepatitis B virus in a number of populations. In tropical Southeast Asia studies at the SEATO Medical Research Laboratory have documented serological evidence of prior HBV infection in up to 75% of Bangkok residents and a carrier prevalence of $HB_s Ag$ of approximately 9% (1). In recent years a large number of Americans, largely military personnel, have been stationed in Southeast Asia. These Americans came from an area in which $HB_s Ag$ is found in only 0.1 to 1.0% of the population and evidence of prior HBV infection in only 5 to 20% (2, 3).

A study of Americans entering and leaving the Republic of Vietnam in 1970 showed that troops arriving for an initial tour and those leaving after approximately one year had had equal experience with hepatitis B virus (Table 1). Troops arriving in Vietnam for a subsequent tour, however, had significantly greater experience with hepatitis B virus. The data suggested that previous experience in Vietnam was related to a greater experience with hepatitis B virus. This study was designed to determine the environmental and host factors which lead to the development of clinical and subclinical hepatitis among American troops in Southeast Asia.

DESCRIPTION: A description of the design of this study appeared in the SEATO Medical Research Laboratory Annual Report 1973–1974. Briefly the population studied was drawn from servicemen aged 18–25 years in grades E1–E5 entering either the United States Army Support Group, Thailand or the United States Air Force 635th Combat Support Group. Shortly after arrival in Thailand a questionnaire was administered to these men to determine, among other things, their previous duty station, previous tropical experience and their prior experience with hepatitis. During the ensuing year these men were interviewed three times at approximately four month intervals. The interviews contained questions of social behavior and medical experience. Serum samples were collected at the time of each interview and were submitted for detection of hepatitis B surface antigen (HB_sAg) by complement fixation (CF), immunoelectrophoresis (IEOP) and radioimmune assay (RIA). Antibodies against hepatitis B surface antigen (anti–HB_s) were detected by a radioimmune assay inhibition (RIAI) and confirmed and titered by a passive hemagglutination test (PHA). Methods for these assays have appeared elsewhere (SEATO Medical Research Laboratory Annual Reports 1971–1972, 1972–1973 and 1973–1974).

PROGRESS: Subjects were enrolled in this study between April and December 1972. Initial questionnaires were completed in December 1972. The first of three follow–up interviews was completed in April 1973, the second in August 1973 and the third in December 1973. The three follow–up interviews and bleeds were divided according to timing into groups. The time of the first interview fell between 12 and 24 weeks (3 and 6 months), the second between 24 and 38 weeks (6–9.5 months) and the last between 39 and 65 weeks (9.5–16 months). Individuals whose interviews fell outside of these time periods were excluded from the study. With these stipulations, there were 418 individuals who completed the

initial questionnaire and from whom blood was obtained. Of these, 385 (92%) were seen at the first follow-up, 317 (76%) at the second and 326 (78%) at the third. Two hundred and seventy-one people (61%) were completely followed with all three interviews and bleeds. Table 2 shows the prevalence of past experience with hepatitis B virus at each bleed and the incidence of infection for the period of time from the first bleed. Table 3 shows similar data for 271 people who were completely followed with all three interviews and four bleeds.

Table 1. Evidence of Hepatitis B Virus Infection in American Military Personnel in the Republic of Vietnam

Military Personnel	HB_sAg	Anti-HB_s
Inprocessing		
Initial tour	0.39% (4/1004)	3.50% (7/200)
Subsequent tour	2.42% (7/289)	11.28% (22/195)
Outprocessing	0.46% (5/1072)	3.1% (6/189)
TOTAL	0.68% (16/2365)	6.0% (35/584)

Table 2. Experience* with Hepatitis B Virus in American Enlisted Men at Three Month Intervals Throughout One Year's Tour in Thailand

Bleed	1st	2nd	3rd	4th
Weeks in country	0	13-25	26-39	40-65
Total number studied	418	385	316	326
Persistent evidence	—	16 (4.2%)	15 (4.8%)	14 (4.3%)
Incidence	—	8 (2.0%)	12 (3.7%)	17 (5.2%)
Prevalence	18 (4.3%)	24 (6.2%)	27 (8.5%)	31 (9.5%)

* Experience is determined by presence of HB_sAg or anti-HB_s.

Table 3. Experience with Hepatitis B Virus in 271 American Enlisted Men Completely Followed Throughout One Year's Tour in Thailand

Bleed	1st	2nd	3rd	4th
Weeks in country	0	13–25	26–39	40–65
Persistent evidence	—	13 (4.7%)	13 (4.7%)	13 (4.7%)
Incidence	—	7 (2.5%)	12 (4.4%)	16 (5.9%)
Prevalence	13 (4.7%)	20 (7.3%)	25 (9.2%)	29 (10.7%)

Three hundred and twenty-six individuals were followed with at least a first and a fourth bleed. At the risk of falsely inflating the prevalence and incidence of hepatitis B infection, six additional individuals were added. These six men were followed with at least two bleeds; three had evidence of prior HBV infection on the first bleed and three developed antibody during the study. Table 4 illustrates the number of HBV infections recorded in these 332 men over the one year period.

Table 4. Hepatitis B Infections Recorded in 332 American Enlisted Men Followed Through One Year's Tour of Duty in Thailand

HBV Serology	Follow–up Blood Sample				
	1st	2nd	3rd	4th	Total
HB_sAg positive	2 (0.6%)	1 (0.3%)	3 (0.9%)	4 (1.2%)	10 (3.0%)
Anti-HB_s positive	16 (4.8%)	7 (2.1%)	5 (1.5%)	5 (1.5%)	33 (9.9%)
Total HBV Experience (HB_sAg + Anti-HB_s)	18 (5.4%)	8 (2.4%)	7*(2.1%)	8*(2.4%)	41 (12.3%)

* Two persons who developed antigen followed by antibody are counted only once in the total HBV experience.

Six clinical cases of hepatitis were diagnosed in these 332 men during the periods between bleeds (Table 5). There were four individuals in whom clinical hepatitis was associated with HBV. No HB_sAg was identified in one of these but anti–HB_s developed in the convalescent period by the time of the second bleed. In the second case, HB_sAg was detected in the second blood specimen at the time of clinical disease and anti–HB_s was found in the third. The two remaining cases were diagnosed in the third period; in both of them HB_sAg was detected in the fourth blood sample. No further follow–up samples were obtained from either of them. There were two cases of hepatitis diagnosed with no detectable evidence of HBV infection. These are listed as HBV non–associated hepatitis; however, these individuals may have had HBV infections which might have been detected by more sensitive tests.

In this group of 332 men, HBV associated hepatitis was diagnosed in four of them. Serological evidence of inapparent infection was documented in an additional 19 men. The incidence of HBV infection over the one year period was 23/332 or 6.9% and the apparent to inapparent infection ratio was 4:19.

In analysing these figures, those men who were entering a tropical area for the first time were compared to those who had prior experience in the tropics. Tables 6 and 7 document the differences seen in these two groups.

Table 5. Clinical Hepatitis Infections Recorded in 332 American Enlisted Men Followed Through One Year's Tour of Duty in Thailand

Type of Hepatitis	Four Month Interval			
	1	2	3	Total
HBV Associated	1	1	2	4
HBV Non-associated	1	1		2
Total Clinical Hepatitis	2	2	2	6

These data have been coded for computer analysis. Cross tabulations of hepatitis experience with variables, such as drug use, mixing with the indigenous population and sexual experience will be computed to determine if hepatitis in these troops is associated with any identifiable behavioral pattern.

DISCUSSION: In a group of 332 young American military personnel followed in Thailand, clinically recognizable hepatitis occurred in six (18/1000) over a one year period. Of these six, four (12/1000) had detectable serological evidence of association with hepatitis B surface antigen, the remaining two did not. Screening of these men for the development of HB_sAg or anti-HB_s revealed an additional 19 who had inapparent hepatitis B infection. Thus for HBV infections the apparent: inapparent ratio was as high as 4:19 or nearly 1:5. In other words, 82% of all HBV infections were asymptomatic.

The men in this study demonstrated a direct relationship between previous tropical experience and prior HBV infection as was found in the earlier study in Vietnam. In men without serological evidence of prior HBV infection, however, the incidence of new infection during the current study period was the same, whether or not they had previous tropical experience.

Information on the association of hepatitis B virus infection with social and physical behavior has not yet been analyzed. It is possible that routes of transmission of hepatitis B virus between indigenous populations and United States military personnel may be demonstrated and ways of preventing this infection may be suggested.

Table 6. Prevalence of Demonstrable Experience with Hepatitis B Virus on Entering Thailand: A Comparison of Those With and Without Prior Experience in a High Prevalence Area

Prior Experience	Number of Men	HB_sAg	Anti-HB_s	Total HBV Experience
Yes	119	2[a] (1.6%)	11 (10.1%)	13 (10.9%)
No	213	0 (0.0%)	5 (2.3%)	5 (2.3%)
Total Population	332	2 (0.6%)	16 (4.8%)	18 (5.4%)

a. One man had HB_sAg with a complement fixing titer of 1:64 and carried it throughout his stay in Thailand. The carrier rate = 0.3%

Table 7. Incidence of HBV Infection During a One Year Tour in Thailand: A Comparison of Those With and Without Prior Experience in a High Prevalence Area

Prior Experience	Number of Men Susceptible	HB_sAg	Anti-HB_s	Total HBV Experience
Yes	105	1 (1.0%)	7 (6.6%)	8 (7.6%)
No	208	5 (2.4%)	10 (4.8%)	15 (7.2%)
Total Population	313	6 (2.0%)	17 (5.4%)	23 (7.3%)

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Continuing Studies of Hepatitis B Antigen Carriers in Thailand

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OBJECTIVE: To compare the age specific point prevalences of hepatitis B surface antigen (HB_sAg) carriers and the HB_sAg subtype distributions in carriers in different parts of Thailand.

BACKGROUND: The presence of HB_sAg or antibody to HB_sAg (anti-HB_s) serves as evidence of prior exposure of hepatitis B virus (HBV). Differences in geographical and environmental status may play an important role in the frequency of HB_sAg carriers and in the distribution of antigen subtypes. This study reports the frequency of HB_sAg carriers found in various parts of Thailand.

DESCRIPTION: HB_sAg carriers were identified using the IEOP test previously described. The method for determining subtypes by the immunodiffusion technique using standard reference antigens and hyperimmune rabbit antisera has also been previously described (SMRL Annual Report 1970-1971). The following well defined populations were sampled and tested:

(a) Thai residents of Khao Yai National Park (KYNP) in northeastern Thailand (1),

(b) Randomly selected Thai residents of an urban housing development in Bangkok (2), and

(c) Thai residents of the village of San Kampang, in a semi-rural area of Chiengmai province in northern Thailand (3).

PROGRESS: Residents of KYNP included the families of employees of three different agencies working in the park: The Forestry Department, the Highway Department and the Tourist Organization of Thailand (TOT). More than 80% of the estimated total population of the park were sampled and the prevalence of HB_sAg carriers was 9.3%. HB_sAg was found in 10.6% of people associated with the Forestry Department, 9.1% in those associated with the TOT and in only 5.3% of the people associated with the Highway Department.

The prevalence of HB_sAg in males was higher than that in females for every age group. The difference in carrier frequencies between males and females was statistically significant for the total population ($P < .001$). The prevalence of HB_sAg was greatest in children between ages of 10 and 15 years; it fell in older age groups. Of 223 people with multiple blood samples, three acquired HB_sAg between September 1973 and September 1974; the incidence of antigen acquisition was 13.5/1000/year.

Comparison of the above results with the prevalence of HB_sAg carriers in the urban Bangkok housing development (Table 2) revealed that the frequencies of HB_sAg carriers were not significantly different in the two populations. However, in this urban group, there was no significant sex differences in the carrier frequencies of HB_sAg ($0.157 > p > 0.317$). Children of five to nine years had a slightly higher frequency of antigenemia than other age groups with the exception of the small group of people over 60 years of age.

In a village population drawn from northern Thailand, the prevalence of HB_sAg was 8.6% (Table 3). This was not significantly different from those found in the populations described above. None of the 51 sera collected from females was found to contain HB_sAg, but it was found in 15% of those collected from males. The significantly higher prevalence of HB_sAg carriers among males than females was similar to that seen in KYNP ($0.008 > p > 0.014$). San Kampang was one of four villages studied in northern Thailand. In the other three, there was no appreciable prevalence of HB_sAg. If all four villages were taken together, the prevalence of HB_sAg fell from 8.6 to 2.9%.

Subtyping of HB_sAg in carriers was studied in 33 residents of KYNP, 48 of Bangkok and 10 of San Kampang (Table 4). Antigens of adr subtype were present in 86% of the Bangkok carriers, 90% of KYNP carriers and 100% of these in the northern Thai village. The findings suggest that within Thailand, there may be differences in the relative frequency of HB_sAg subtypes from place to place.

Several recent studies have shown that subtypes are consistent within families; however, this information was obtained in the temperate zone where antigen carriers are rare and contact by family members with antigens other than those carried in the family would be unlikely. In KYNP, different subtypes of antigen were present in close proximity. Five conjugal families were studied in which the antigen carried by at least two positive individuals could be subtyped, in four families only the adr subtype was identified and only the adw subtype was found in the other family. Only one subtype was found in any one family. Within the urban Bangkok population, the distribution of subtype again fit into family patterns. In four conjugal families with two to seven HB_sAg positive members, only the adr subtype could be detected; in two other families with two and four HB_sAg positive members, respectively, only the adw subtype was found. Again in no family was more than one subtype identified (4).

Table 1. Age Specific Prevalence of HB_sAg in Residents of Khao Yai National Park
(September 1973 – September 1974)

Age (Years)	Male			Female			Total		
	No. Tested	HB_sAg+ No.	(%)	No. tested	HB_sAg+ No.	(%)	No. Tested	HB_sAg+ No.	(%)
0–4	27	1	(3.7)	35	0	(0.0)	62	1	(1.6)
5–9	27	3	(11.1)	26	0	(0.0)	53	3	(5.7)
10–14	14	5	(35.7)	13	0	(0.0)	27	5	(18.5)
15–19	36	3	(8.3)	18	0	(0.0)	54	3	(5.6)
20–29	130	20	(15.4)	57	3	(5.3)	187	24	(12.8)
30–39	60	7	(11.7)	18	1	(5.6)	78	8	(10.3)
40–59	27	3	(11.1)	8	0	(0.0)	35	3	(8.6)
60+	1	0	(0.0)	0	0	(0.0)	1	0	(0.0)
Total	322	42	(13-0)	175	4	(2.3)	497	46	(9.3)

HB_sAg Acquisition Rate = 3/223/year = 13.5/1000/year

Table 2. Age Specific Prevalence of HB_sAg* in Residents of Huay Khwang, Bangkok (July 1971)

Age (years)	Male			Female			TOTAL		
	No. tested	Prevalence No.	%	No. tested	Prevalence No.	%	No. tested	Prevalence No.	%
1–4	35	1	(2.8)	31	2	(6.4)	66	3	(4.5)
5–9	54	5	(9.2)	56	7	(12.5)	110	12	(10.9)
10–14	61	8	(13.1)	66	2	(3.0)	127	10	(7.9)
15–19	39	6	(15.4)	54	3	(5.6)	93	9	(9.7)
20–29	38	5	(13.2)	71	4	(5.6)	109	9	(8.2)
30–39	27	0	(0.0)	53	4	(7.5)	80	4	(5.0)
40–59	37	2	(5.4)	56	3	(5.4)	93	5	(5.4)
60+	6	2	(33.3)	13	3	(23.1)	19	5	(26.3)
TOTAL	297	29	(9.8)	400	28	(7.0)	697	57	(8.2)

* Combined results from IEOP and radioimmunoassay(RIA) tests.

Table 3. Age Specific Prevalence of HB_sAg in San Kampang (November 1969)

Age (Years)	Male			Female			Total		
	No. Tested	HB_sAg+ No.	(%)	No. Tested	HB_sAg+ No.	(%)	No. Tested	HB_sAg+ No.	(%)
0–4	3	1	(33.3)	8	0	(0.0)	11	1	(9.1)
5–9	15	4	(20.6)	9	0	(0.0)	24	4	(16.7)
10–14, 15–19	21	2	(9.5)	15	0	(0.0)	36	2	(5.9)
20–29, 30–39	15	3	(20.0)	11	0	(0.0)	26	3	(11.6)
40–59, 60+	11	0	(0.0)	8	0	(0.0)	19	0	(0.0)
Total	65	10	(15.4)	51	0	(0.0)	116	10	(8.6)

DISCUSSION: Comparison of the three populations revealed no significant differences in the frequency of HB_sAg carriers, suggesting a similar rate of exposure to HBV occurred in these three groups. It was interesting to note, however, the marked local differences in the antigen frequency among the villages of northern Thailand. On the one hand, the number of persons studied were small and therefore the phenomena may represent a sampling error. On the other hand, these differences may be due to local environmental and social factors. The high prevalence of HB_sAg carriers among all three groups support the finding of high carrier prevalences in the tropics. The striking difference noted in the HB_sAg carrier prevalence among the rural males and females might be explained by two hypotheses. First the male, through his socially defined role, may have an increased exposure to sources of infection. This has been suggested by the data of Grossman et al (2) who showed increased anti $-HB_s$ prevalences among young urban males. The distribution of anti $-HB_s$ in rural populations remains to be seen. Second, males may be more susceptible than females to the development of the carrier state. This interesting hypothesis could not be evaluated in this study.

The subtype distribution in carriers suggests that local differences in virus subtypes exist within areas of Thailand. Further, the localization of antigen subtypes within family units implies an even smaller unit of HBV transmission.

Table 4. HB_sAg Subtypes in Thais

Population	HB_sAg adr %	Subtypes adw %
Urban Bangkok	86	14
Khao Yai National Park	90	10
Northern Thailand	100	0

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The Effect on the Offspring of Maternal Hepatitis B Surface Antigenemia

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OBJECTIVE: To study the effect on the offspring of chronic hepatitis B antigenemia in the mother.

BACKGROUND: Clinical hepatitis B developing during the latter part of pregnancy has been associated with an increased perinatal mortality, a high incidence of premature delivery and a high frequency of virus transmission from mother to infant (1). Information on the effect of asymptomatic maternal hepatitis B antigenemia is conflicting. It has been suggested that the incidence of prematurity and perinatal mortality is also increased in infants born of these mothers (2). Transmission of virus from antigenemic mothers to their infants appears to be an uncommon event in mother infant pairs studied in the West (3, 4). However, a strong association between antigenemia in mothers and their children has been shown in cross sectional population studies in the Far East (5, 6). This study was designed to investigate the effect of maternal antigenemia on pregnancy and the offspring in a population with a high prevalence of antigen carriers.

DESCRIPTION: This study was divided into two phases. A description of the initial phase may be found in the SEATO Medical Research Laboratory Annual Progress Report, March 1974.

Population: Antigen positive mothers and their families were sought for follow-up $1\frac{1}{2}-2\frac{1}{2}$ years after initial collection in the delivery room. Temporal controls were matched to each family that could be located. Temporal controls were members of families of women who were collected in the delivery room and whose delivery dates were as close as possible to those of the antigen positive mothers. Each control mother was delivered within two days of an antigen positive mother. Interim family histories were obtained on all families studied and these included the duration of breast feeding, the medical and dental history of the child and the person who cares for the child most of the time. Family relationships were determined and other members of the family living in the household were sought. Each infant was examined by a physician and height and weight measurements were recorded.

Sera were collected on antigen positive and control mothers, their infants, and as many family members as could be found. Saliva was also collected from all mothers.

Laboratory Studies: Sera were submitted for determinations of transaminase and bilirubin concentrations. They were tested for hepatitis B antigen by radioimmune assay (Abbott Laboratories, Ausria I), counterelectrophoresis and complement fixation. A radioimmune assay inhibition technique was used to screen for antibody against HB_sAg and positives were titrated and confirmed by a passive hemagglutination test (PHA, Electronucleonics).

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PROGRESS: As reported in the SEATO Medical Research Laboratory Annual Report 1973–1974, of 1,625 mothers screened in the delivery room at Women's Hospital, 93 or 5.7% were found to be positive. Of the 93, 47 were located, 30 were not located and 16 lived outside of Bangkok.

Ninety–four families were followed, 47 with antigen positive mothers and 47 temporal controls. There were no significant differences between the antigen positive family and the temporal control found in family variables such as household size and income. Five families of antigen positive mothers who resided outside of Bangkok, returned to the city for follow–up. If these five were discounted, then the distribution within the city of the families of antigen positive mothers and controls was similar. Maternal factors such as age, parity, history of past abortion, infant mortality and transaminase levels also showed no significant differences. Further, there were no significant differences seen in the weight, length or transaminase levels of the infant at birth, nor in the number of infants born prematurely or the infant mortality rate over the first year of life. In the 94 families followed, five infants had died. Two deaths were recorded within the control families and the other three in families of antigen positive mothers. Three of these deaths occurred at or shortly after delivery. Two were related to complications of delivery and one to prematurity. Two children died during the first year of life. One child of an antigen positive mother died at four months of age during an episode of diarrhea for which no medical aid was sought. A child of a control mother died of pneumonia at six months of age.

Table 1. Experience with Hepatitis B Virus 18–30 Months After Birth

Mother	Offspring			
	No. Tested	Evidence of Infection		
		HB_sAg	Anti–HB_s	Total
HB_sAg Positive	44	13 (30%)	3 (7%)	16 (37%)
HB_sAg Negative	45	0 (0%)	1 (2%)	1 (2%)

Table 2. Maternal Antigen Titer and the Percent of Positive Offspring

Mothers		Offspring	
CF Titer	No. Tested	HBsAg Positive	
		No.	%
≤1 : 16	60	0/60	0
1 : 32	7	1/7	14
1 : 64	12	5/12	42
> 1 : 128	10	7/10	70
Total	89	13/89	14

All mothers who were antigen positive at delivery were still positive at the time of follow–up 1 ½ to 2 ½ years later. In general the complement fixation titers of these mothers were within four–fold of the titer found at delivery. None of the temporal control mothers or their infants had developed antigen; however, 13 of 44 (29,5 %) of surviving infants of antigen positive mothers were found to be positive (Table 1).

Despite what would appear to be abundant hepatitis B virus exposure in infants of HB_sAg positive mothers, the incidence of antibody conversion in this group was low (Table 1). There were no significant differences in the incidence of antibody conversion in infants with positive mothers and in those of control mothers; however, there were three times as many converts in the antigen positive group and the lack of significance might reflect only the small number of infants examined.

There appeared to be a direct relationship between the maternal complement fixation titer at delivery or follow–up and the prevalence of antigen in the serum offspring for follow–up (Table 2). The maternal titer on all 13 infants found to be positive was greater than or equal to 1:32. None of the offspring of mothers with titers less than 1:32 were positive.

At the time of follow–up, the physical status of children of antigen positive mothers and antigen negative controls was documented. None of these children were chronically ill, nor with one exception did any exhibit any biochemical or physical evidence of hepatitis at the time of examination. When compared to normal values established for Southern Chinese children (7), average deviations of height and weight for these two groups of children were not significantly different. The one exception was the antigen positive son of an antigen positive mother whose only sign of illness was a moderate elevation in the transaminase concentrations (SGOT to 136 Sigma Frankel units, SGPT to 90 Sigma Frankel units).

DISCUSSION: As reported in the SEATO Medical Research Laboratory Annual Report 1973–1974, there were no gross differences seen in the prematurity and perinatal mortality rates of infants of antigen positive mothers and antigen negative mothers. Further, there were no apparent differences in the maternal history of pregnancy and child birth between these two groups; however, subtle differences could not be excluded.

Transmission of hepatitis B virus occurred from mother to offspring. In this study only infants of positive mothers were found to be positive at 1 ½ to 2 ½ years of age. This does not exclude the possibility of virus transmission and development of the carrier state in children of antigen negative mothers; indeed this must happen in order to maintain the high prevalence of antigen carriers seen in this population. These data do suggest that the infection of infants of negative mothers is a relatively rare event when compared to that of children of antigen positive mothers. The phenomenon of hepatitis B virus transmission appears to be directly related to the antigen titer of the positive mothers. The prevalence of antigen positive offspring increased as the HB_sAg titer in the mother increased.

Maternal antigenemia did not grossly effect the growth or the development of the child. Children who developed HB_sAg were not significantly different in height and weight from children without HB_sAg, whether or not they had HB_sAg positive mothers.

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Hepatitis B Virus in Bangkok Families

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OBJECTIVE: To determine when young urban Thai children are first exposed to hepatitis B virus (HBV) and to search for the most common routes of transmission to infants in the first year of life.

BACKGROUND: A recent study showed that 19.9% of the residents of Huay Khwang had Hepatitis B surface antigen (HB_sAg) or antibody (anti–HB_s) between the ages of one to five years (1). HBV infection in children was closely related to the presence of HB_sAg in their mothers. Another study of women who delivered at Women's Hospital, Bangkok, showed 12% of 93 mothers with HB_sAg had antigen in their cord blood by radioimmune assay (RIA). Furthermore, mothers with anti–HB_s always had antibody in their cord bloods (2).

The current study was designed to follow infants in the first year of life and to compare the incidence and effects of HBV infection in infants whose mothers had HB_sAg or anti–HB_s to those whose mothers were negative.

DESCRIPTION: An attempt was made to interview and sample as many women as possible who delivered at Phra Mongkutklao Hospital (PMKH) between 1 February 1974 and 31 January 1975. Blood was collected from the mother and the carefully wiped umbilical cord at the time of delivery for testing for HB_sAg and anti–HB_s. A questionnaire interview of the mother was conducted in the early postpartum period. Study subjects were selected by 1) the presence of HB_sAg or anti–HB_s in the mother's blood; 2) residence within the metropolitan Bangkok area; and 3) willingness to allow home visits and to bring the baby to the PMKH Well Baby Clinic for follow–up. Control mothers were selected if they had no HB_sAg or anti–HB_s in their blood but delivered on the same day as a positive mother. Control mothers also had to meet criteria 2 and 3 listed above.

HBV serology used a solid phase RIA (Ausria I) and immunoelectroosmophoresis (IEOP) as the primary screening tests for HB_sAg and a radioimmune assay inhibition (RIAI) test to detect anti–HB_s. Passive hemagglutination (PHA) was used when available to confirm the RIAI results and to test small volume samples. All blood samples were tested for serum transaminase (SGOT and SGPT) levels as well.

Serial serum samples were drawn by venipuncture at approximately two, three, six, nine and 12 months of age in the Well Baby Clinic after examination by a pediatrician. Blood samples were drawn from the mothers at the same time intervals. An attempt is being made to collect blood samples from all other people living in the home during home visits at three, six and 12 months after delivery.

During home visits, information was gathered on the home environment by questionnaire and inspection and samples of breast milk, saliva and mosquitoes were collected from some families. The priority of sample testing is to test sera first, then saliva, breast milk and mosquitoes.

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PROGRESS: A comparison was made of 300 women delivering at PMKH to 300 women at Women's Hospital to see if the two hospital populations were similar. The prevalence of HB_sAg detected by IEOP was 4.3% at PMKH and 3.7% at Women's Hospital. The groups were very similar in terms of parent ages, number of people in the home and home location within Bangkok. A notable difference was that the mean family income was 25% greater at PMKH than at Women's Hospital. For the purposes of this study, the two hospitals seemed similar.

Interviews and blood samples were obtained from 1042 (43%) of the women who delivered over a 12 month period. From this group, 42 women with HB_sAg, 44 with anti-HB_s and 77 negative controls are being followed. For most mothers with antigen or antibody, a satisfactory negative control was identified who delivered two days before to two days later. In seven instances, mothers who were initially thought to be negative were later shown to actually have antibody at the time of delivery after they had been matched to HB_sAg positive mothers. These seven pairs of antigen positive mothers mismatched to antibody positive mothers are being followed that way.

A preliminary review was made of the serological results for 64 mothers, including 12 with HB_sAg, 20 with anti-HB_s and 32 time matched controls. Only families that had been followed at least six months or seroconverted before being lost to follow-up were reviewed. All mothers with antigen or antibody remained positive (Table 1). Three negative mothers developed low level antibody activity by 10-19 weeks suggesting they may have been exposed to HBV in the recent past.

The infants showed dramatic serological changes (Table 2). One infant of an HB_sAg positive mother was found to have antigen in the cord blood and in every follow-up serum throughout the next 12 months. This infant had the only antigen positive cord blood detected by IEOP. Other infants of antigen positive mothers frequently developed antigen or antibody by the age of 20-29 weeks, indicating that these infants are at risk of infection very early in life.

All of the infants with antibody positive mothers had anti-HB_s in their cord bloods. The frequency of anti-HB_s declined steadily during the first six months as was expected for passively acquired maternal antibody. Several of these infants came from families with an HB_sAg positive father, sibling or other member; some may show evidence of infection with HBV after the maternal antibody is gone. None of the infants of negative mothers have developed antigen or antibody yet. The prevalence of HB_sAg carriers seems to be the lowest in this group of families.

SUMMARY: A prospective study was started of HBV infection of infants selected on the basis of their mother's serological findings at the time of delivery. A preliminary review indicates children of HB_sAg positive mothers have a high likelihood of becoming infected in the first six months of life. Children of antibody positive mothers have maternal antibody at birth which may afford protection during the first six months. After losing their maternal antibody, these children as a group may be at a higher risk of infection with HBV than children of negative mothers since the families of the former often include an antigen carrier.

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Table 1. Frequency of HB_sAg and Anti-HB_s in 32 Pairs of Mothers.

Mother		Maternal Category								
Blood Spec.	Weeks After Delivery	HB_sAg			Anti-HB_s			Negative		
		No.	Ag+	Ab+	No.	Ag+	Ab+	No.	Ag+	Ab+
1	Delivery	12	12	0	20	0	20	32	0	0
2	4 – 9	8	8	0	16	0	16	24	0	0
3	10 – 19	9	9	0	18	0	18	27	0	3
4	20 – 29	7	7	0	14	0	14	21	0	2
5	30 – 39	2	2	0	1	0	1	3	0	0

Table 2. Frequency of HB_s Ag and Anti-HB_s in 32 Pairs of Infants.

Infant		Maternal Category								
Blood Spec.	Weeks After Delivery	HB_sAg			Anti-HB_s			Negative		
		No.	Ag+	Ab+	No.	Ag+	Ab+	No.	Ag+	Ab+
1	Delivery	12	1	0	20	0	20	32	0	0
2	4 – 9	8	0	0	16	0	15	24	0	0
3	10 – 19	9	3	1	18	0	10	27	0	0
4	20 – 29	7	4	1	14	0	2	21	0	0
5	30 – 39	2	1	0	1	0	0	3	0	0

Hepatitis B Surface Antigen in Laboratory Reared Mosquitoes

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OBJECTIVE: To determine the duration of carriage of hepatitis B surface antigen (HB_sAg) by laboratory reared mosquitoes fed on a HB_sAg carrier.

BACKGROUND: This study was reported in the SEATO Medical Research Laboratory Progress Report 1973—1974. This report concerns the completion of radioimmune assay testing of mosquitoes following feeding on an antigen positive donor.

DESCRIPTION: All mosquitoes used in this study were reared from eggs in the laboratory. After the adults emerged they were held for 48 hours and were deprived of fluids for 12 hours prior to use. Mosquitoes were fed on a known carrier of HB_sAg/adr with a constant complement fixation titer of 1:512. Engorged mosquitoes were then removed and unfed mosquitoes discarded. A sample of 10 fed mosquitoes were quick—frozen and stored at —70°C; the remainder were placed in cages and allowed to feed on sugar water. Samples of 10 mosquitoes were withdrawn from the cages at 1, 3, 5, 7, 10, 15 and 21 days after feeding, quick—frozen and stored at —70°C.

All mosquitoes were tested by radioimmune assay (RIA, Ausria I, Abbott Laboratories) simultaneously for each mosquito species. Pools of 10 mosquitoes were triturated in 0.5 ml of 0.01 M Tris buffered saline pH 7.4 and centrifuged at 2000 rpm; 0.1 ml of the supernatant solution was placed in each of two Ausria tubes. Following this the test was run according to the directions provided with the Ausria kit. Included in each experiment was a pool of 10 unengorged mosquitoes of each species. Also one mosquito species, *Aedes aegypti*, was allowed to bite a non—antigenemic individual. These mosquitoes were followed in the same way.

PROGRESS: Seven mosquito species, *Aedes aegypti, Aedes albopictus, Anopheles balabacensis, Anopheles maculatus, Anopheles minimus, Armigeres subalbatus,* and *Culex quinquefasciatus,* were tested in the above manner (Figure 1). RIA results of all unengorged mosquito controls fell within one standard deviation of the mean of the negative sera controls. Further, all samples of *Aedes aegypti* fed on a non—antigenemic individual also were found to fall within one standard deviation of the negative control mean. For all seven species the first pool of ten was taken immediately after feeding on the HB_sAg positive volunteer. HB_sAg was detected in all mosquito species in the first sample. However, HB_sAg as determined by RIA, had disappeared by 24—72 hours after feeding. All mosquito species were followed for 21 days or longer. In all species HB_sAg did not reappear; the RIA counts per minute on these mosquito pools remained within the limits of the unfed mosquitoes.

DISCUSSION: These data indicate that disappearance of HB_sAg from the mosquito pools was simultaneous with the digestion and elimination of the blood meal. In the seven mosquito species followed for 21 or more days HB_sAg did not reappear after its initial disappearance; however, the presence of antigen in mosquitoes for 24—72 hours might allow them to serve as mechanical vectors if they refeed within this period of time.

The laboratory work on this study is now complete and the data is being analysed in preparation for publication.

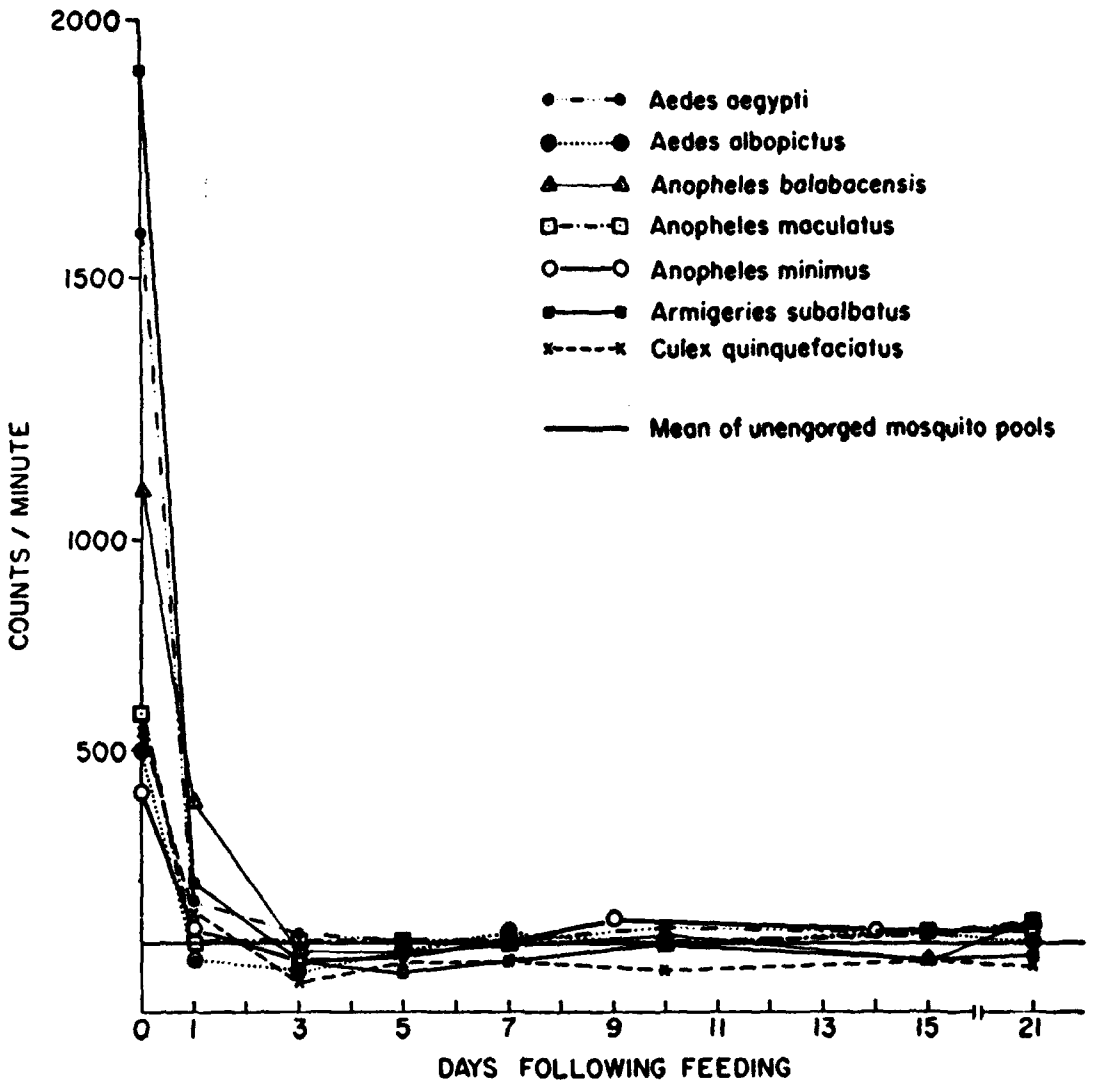

Figure 1 Radioimmuno assay for HB_sAg (Ausria 1) on pools of 10 mosquitoes collected following feeding on an infectious HB_sAg positive volunteer.

Tick–borne Viruses in Thailand

1. Tentative Identification of Langat Virus

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OBJECTIVE: To identify a group B arbovirus (T–1674) isolated from ticks in Khao Yai National Park.

BACKGROUND: Three unidentified viruses have been isolated from ticks collected in Thailand (1). One of the viruses, T–1674, was shown to pass through a 200 nm filter. Growth was inhibited by either exposure to ether or pH 3.0 but not by a DNA inhibitor, 5–Bromo 2' deoxyuridine. In LLC–MK_2 cells, T–1674 produced plaques of varying size. Complement fixation (CF), hemagglutination inhibition (HI) and plaque reduction neutralization tests (PRNT) indicated T–1674 was antigenically related to the group B arboviruses. Tick–borne group B arboviruses have not been previously identified in Thailand. Since some members of this group cause severe encephalitis in man, identification of T–1674 was given first priority.

DESCRIPTION: Identification of T–1674 was accomplished by comparing its antigenicity to that of nine other group B arboviruses including a prototype strain of Langat, TP–21.

Hyperimmune mouse ascitic fluid (HMAF) was used in all serological tests. HMAF was made to T–1674 and the prototype Langat TP–21 (2). Additional HMAF to TP–21 was kindly provided by Dr. Hazel Wallace (Arbovirus Research Unit, University of Malaya, Kuala Lumpur, Malaysia).

CF and HI antigen was prepared by sucrose acetone extraction of suckling mouse brain (SMB) (3). Hemagglutin activity was optimal at pH 6.7 (range 6.4–7.0) at 22°C. PRNT using a constant amount of virus and dilutions of HMAF were used to determine the dilutions of antibody giving 50% plaque reduction (4).

PROGRESS: Low passage seed virus sent to the Yale Arbovirus Research Unit, New Haven, Conn., was tentatively identified as Langat virus by comparative CF testing (5). Similar testing in this laboratory supported this conclusion (Table 1). In addition, PRNT showed T–1674 was neutralized only by Langat antibody (Table 2). Although the Langat HMAF gave two to four fold higher antibody titers to TP–21 than to T–1674, it is concluded that there is sufficient similarity to consider T–1674 to be a new strain of Langat virus.

SUMMARY: Identification tests of a group B tick–borne virus (T–1674) from Khao Yai National Park suggest it is a new strain of Langat virus. Conclusive identification awaits interpretation of the results by the Yale Arbovirus Research Unit.

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Table 1. Comparative Reciprocal CF Antibody Titers to Four Units of Antigen

Antigen	Hyperimmune Mouse Ascitic Fluid*									
	T–1674	Langat	Dengue 1	Dengue 2	Dengue 3	Dengue 4	Japanese Encephalitis	Wesselsbron	Tembusu	West Nile
T–1674	32	256	2	<2	<2	<2	4	<2	<2	<2
Langat (TP 21)	32	256								
Dengue 1	<2		512							
Dengue 2	<2			1024						
Dengue 3	<2				512					
Dengue 4	<2					512				
Japanese Encephalitis	<2						512			
Wesselsbron	<2							256		
Tembusu	<2								64	

* HMAF were not absorbed with normal mouse brain.

Table 2. Comparative Reciprocal PRNT Antibody Titers to 95 Pfu of Virus

Virus	Hyperimmune Mouse Ascitic Fluid							
	T–1674	Langat (TP21)	Langat (University of Malaysia)	Dengue 2	Japanese Encephalitis	Wesselsbron	Tembusu	West Nile
T–1674	100	600	500	<10	<10	<10	<10	<10
Langat (TP21)	110	2000	1000					

Tick – borne Viruses in Thailand

2. Experimental Infection of Gibbons with a Group B Arbovirus (T – 1674)

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OBJECTIVES: 1) To determine if a group B tick – borne arbovirus (T – 1674) was infectious for gibbons and, if so, 2) to identify any evidence of illness that might also occur in man.

BACKGROUND: Tick – borne group B arbovirus infections of man may be asymptomatic or cause mild to severe encephalitis. A new strain of group B arbovirus, T – 1674, which was isolated from *Haemaphysalis papuana* ticks in Khao Yai National Park (1) was tentatively identified as Langat virus. Langat virus, a member of this group, has been shown to be infectious for rhesus, cynomologus and spider monkeys but not to cause disease. Experimental infection of gibbons has not been reported. Since gibbons are present in abundance in the area in which T – 1674 was found, it is possible that gibbons may be a natural host for this virus.

DESCRIPTION: Three adult gibbons, *Hylobates lar*, which had been cared for by the Dept of Veterinary Medicine, SEATO Medical Research Laboratory for 8 to 9 years, were selected for experimental infection after determining they had no detectable antibody by hemagglutination inhibition (HI) tests to Langat and 5 other group B arboviruses. Two animals (P5, B66s) were inoculated intracutaneously with 1.0 ml each of a low passage suckling mouse brain (SMB) suspension containing T – 1674 at a titer of $10^{4.1}$ suckling mouse LD50/ml. One of these gibbons (B66s) had a splenectomy 7 years previously. The third animal (B56) was inoculated with a placebo and housed in a cage between the infected gibbons. Each animal was observed daily for evidence of illness. Blood samples were collected daily for the first 10 days, then approximately weekly for the first month. Viremia was detected by inoculation of plasma into suckling mice.

PROGRESS: No differences were observed between the three animals with regard to food and water intake, rectal temperature and general behavior that were not within the range of expected daily variation. None of the animals developed a detectable rash, neurological abnormalities, lymph node enlargement or splenomegaly.

Both gibbons who received T–1674 had viremia from days one to six after inoculation; plasma virus titers reached 10^3 suckling mouse LD_{50}/ml on day two and three, respectively. Both animals subsequently developed antibody detected by complement fixation (CF), HI and plaque reduction neutralization test (PRNT) after day 10 (Figure 1). The control gibbon did not develop viremia or detectable antibody. Routine hematological studies showed a temporary fall in hematocrit for each animal during the 10 day period of daily blood collection (Figure 2) which was attributed to frequent phlebotomy. B66s had a long previous record of leukocyte counts above 8000 wbc/ml following splenectomy. The two infected gibbons developed increased total lymphocyte levels relative to their baseline values from days 3 to 10 and 4 to 17, respectively. In these animals, mononuclear cells accounted for 58–70% and 82–88%, respectively, of all leukocytes from days four to nine after infection. In contrast, the control gibbon had 4000 lymphocytes/ml or less during the same time period and 44–63% mononuclear cells.

SUMMARY: Two adult gibbons were experimentally infected with T–1674, a group B arbovirus tentatively identified as Langat virus. Both animals developed viremia from days one to six after infection followed by antibody detected by CF, HI and PRNT. Peak antibody levels were obtained one month after infection. Both animals developed relative and absolute lymphocytosis during the first three weeks after infection but neither developed overt disease. A temporary fall in hematocrit was also seen in the infected and the control animals and was attributed to frequent phlebotomy. It was concluded that T–1674 can cause asymptomatic infections with viremia in gibbons. No additional information was learned about the potential pathogenicity of T–1674 for man.

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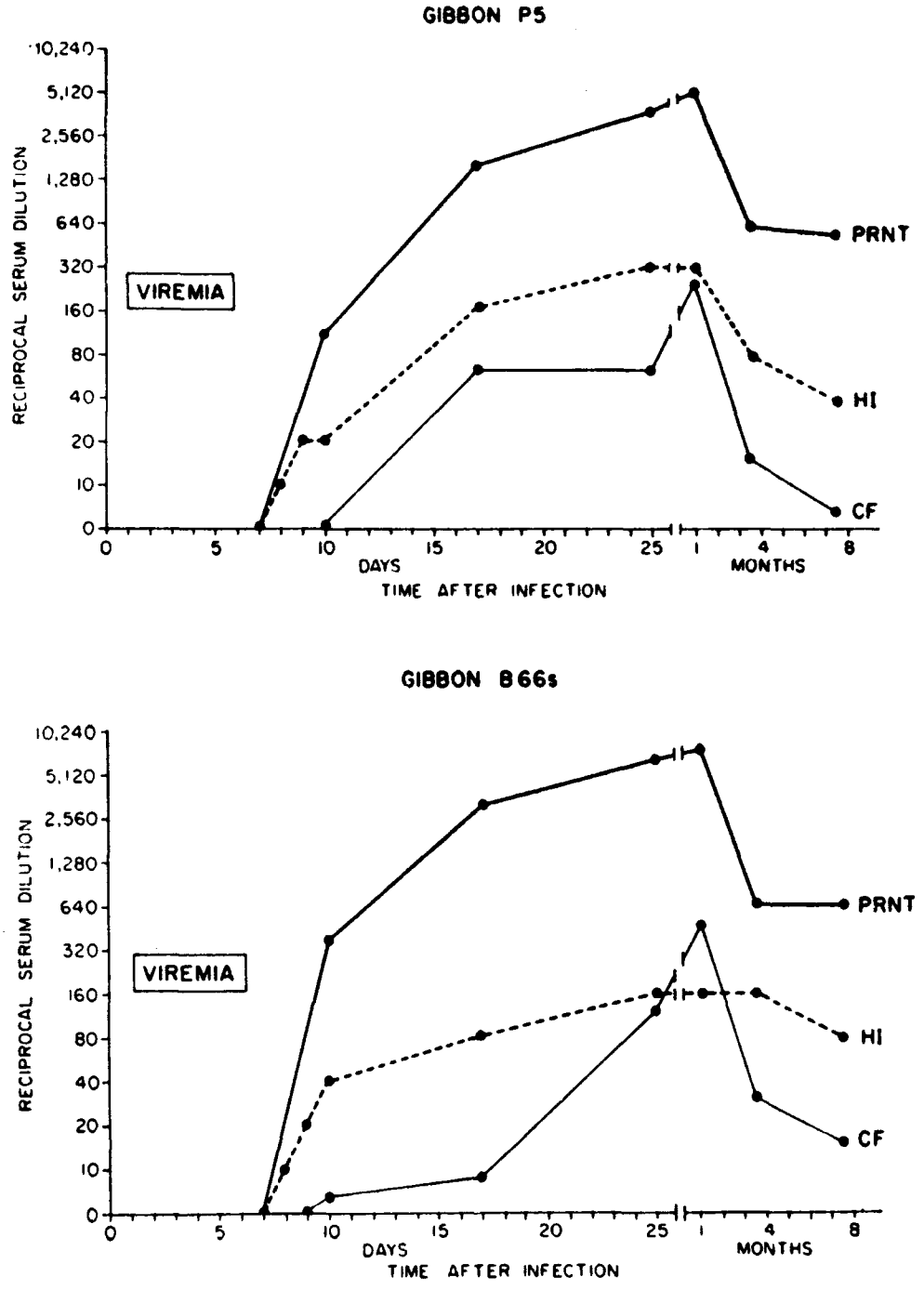

Figure 1 Response of two gibbons to intracutaneous inoculation of $10^{4.1}$ suckling mouse LD_{50} of T-1674 on day 0

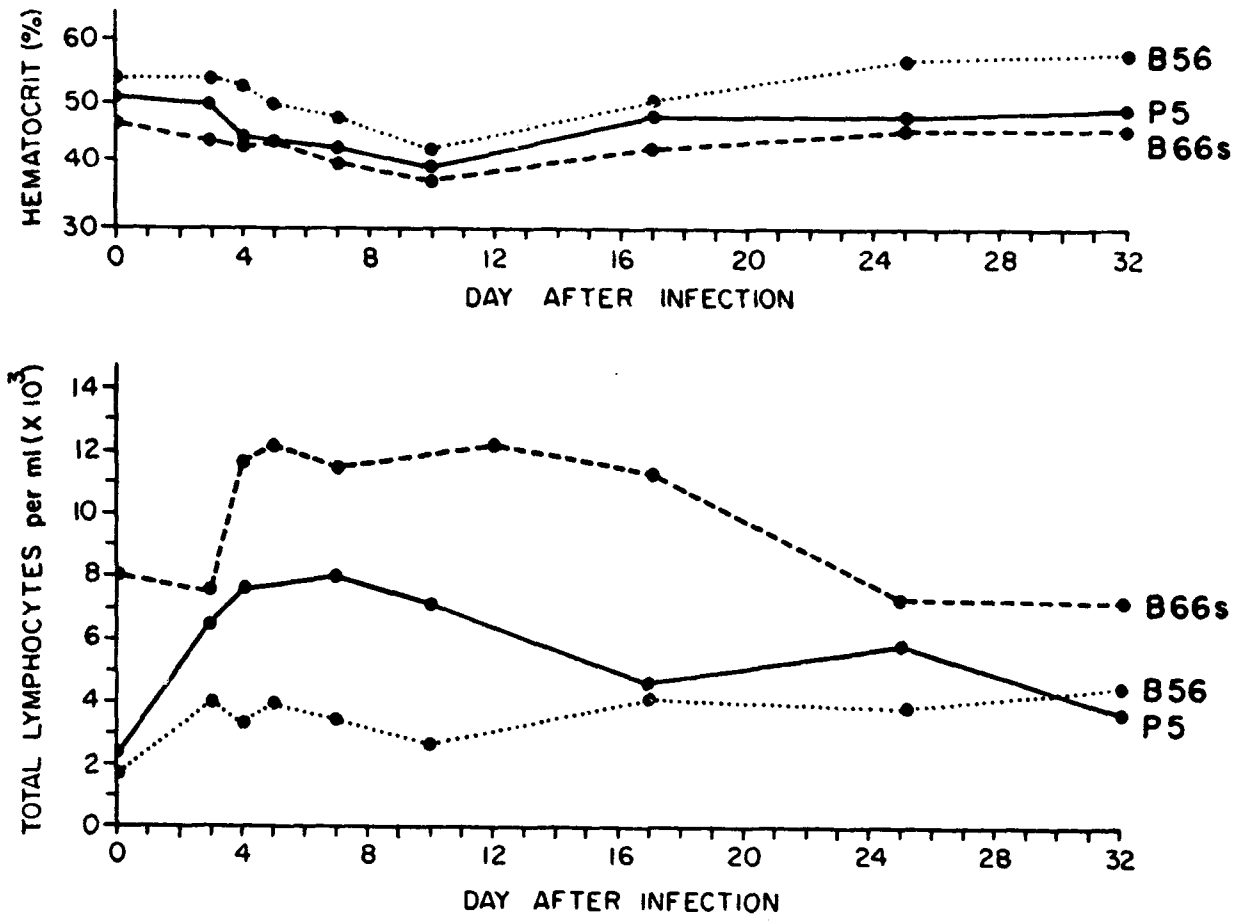

Figure 2 Serial hematocrit and total lymphocyte counts for two gibbons infected with Langat Virus (P5, B66s) and one control (B56). Infection began on day 0.

Tick–borne Viruses in Thailand

3. A Survey for Human Antibody to T–1674

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OBJECTIVE: To detect any evidence of human infection with T–1674, a group B tick–borne arbovirus.

BACKGROUND: Tick–borne arbovirus infections of humans have not been recognized to occur in Thailand. The discovery of two different viruses in ticks collected in Khao Yai National Park (KYNP) stimulated the initiation of a survey of residents of the park for antibody to these agents (1). One of the viruses, T–1674, was previously shown to be a group B arbovirus (1) and is now tentatively identified as Langat virus. Natural infections of humans with Langat is infrequent in Malaysia, but induced infections of people with neoplastic disease has caused encephalitis (2). This study sought evidence of natural human infection with T–1674 in a human population with a high incidence of mosquito–borne group B arbovirus infection.

DESCRIPTION: Human sera were collected from as many residents of Khao Yai National Park (KYNP) as was possible during visits in September 1973, February 1974 and September 1974. At the same time historical information was obtained on the length of residence in KYNP, living site, size of families, occupation, general health and exposure to ticks.

Complement fixation (CF), hemagglutination inhibition (HI) and plaque reduction neutralization tests (PRNT) were used to detect antibody (1). CF antigen was standardized by block titration against homologous HMAF. The highest dilution of a sucrose acetone extract of suckling mouse brain (SMB) giving 50% hemolysis was considered one unit of antigen. A four–fold lower dilution of antigen (4 units) was used in routine CF tests. All sera were heated to 56°C for 30 minutes before testing. A serum titer of 1:4 or greater was considered positive by CF. Eight units of sucrose acetone extracted SMB were used as antigen in HI tests. Positive serum titers by HI were 1:10 or greater. PRNT titers were based on 50% reduction of the mean number of control plaques by a serum dilution of 1:10 or greater.

PROGRESS: Residents of KYNP included employees of the Forestry Department, Highway Department and the Tourist Organization of Thailand (TOT) and their dependent relatives. Between September 1973 and September 1974, serum was collected from 497 individuals representing 80% of the total population estimated from work rosters and interviews and multiple sera were obtained from 39% (Table 1). The median age of the people sampled was 22 years compared to 21 years for the entire population. The ages of people sampled ranged from 4 months to 60 years. The median length of residence in the park was 3 years and ranged from one day to 43 years. The ratio of males to females in the sample was 1.56 compared to 1.39 for the whole population.

The questionnaire survey yielded little evidence of illness. Between 12–55% of the residents experienced one or more of 11 specific symptoms, but the responses did not correlate with the presence or absence of group B arbovirus HI antibody. In September 1973, few people reported ever being bitten by ticks;

however, in February 1974 over 50% of the residents admitted to tick bites and many said ticks were abundant at that time. It appeared that human exposure to ticks was common and probably seasonal.

HI tests were done on at least one blood sample from 488 individuals with adequate demographic information. Of the entire sample, 246 people provided serial blood specimens. The age distribution of the follow-up group was representative of the larger group (Figure 1). Similarly, the age specific prevalence of arbovirus HI antibody for the follow-up group (Figure 2) was representative of all of the park residents. HI antibody to dengue virus type 2 (D2) and Japanese Encephalitis virus (JE) was found in over 65% of persons aged 10-15 years and over 90% after age 20 years. Antibody to T-1674 tended to appear later than that to the other group B arboviruses, was not found in more than 87% of any age group and declined in the oldest age group. Chikungunya antibody was found in only two of 66 persons under 15 years of age but thereafter increased steadily to age 50 years.

Of the 246 residents of KYNP from whom two or three serum samples were obtained, 24 (10.2%) demonstrated a four-fold rise in HI antibody titer to one or more arbovirus antigens (Table 2). A rise in antibody to JE antigen was more frequent than to any other type and was found in individuals ranging in age from 16 months to 54 years. Two people showed a four-fold rise in antibody to T-1674 but both had pre-existing group B antibody. No one developed a higher titer of antibody to T-1674 than to either D2 or JE. The people with multiple serum samples were screened for PRNT antibody at a 1:10 dilution. The highest titer of neutralizing antibody to T-1674 in any resident was 1:10. Since the PRNT is considered to be more specific than the HI test the evidence suggests the HI reactivity to T-1674 was due to cross reactive antibody to other group B arboviruses. The constant presence of mosquito-borne group B arboviruses in KYNP, the low level and infrequent rises in HI antibody to T-1674 and the absence of high levels of PRNT antibody to T-1674 indicate that infection of residents with T-1674 virus is quite infrequent if it occurs at all.

SUMMARY: A prospective survey of arbovirus antibody was made from September 1973 to September 1974 of all residents of Khao Yai National Park. Although evidence was found of near uniform exposure to Dengue 2, Japanese Encephalitis and Chikungunya, there was no conclusive evidence of any natural infection with T-1674 during that time period. It is concluded that HI reactivity to T-1674 antigen is probably due to the presence of cross reactive antibody produced to other group B arboviruses. There appears to be little or no risk of human infection with T-1674 in the park.

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Table 1. KYNP Residents: Serological Sampling
Sept 1973 – Sept 1974

Dept. Group	Residents No. (%)	Sex Male	Female	M/F	Age* Median	(Range)	Length of Residence* Median	(Range)
TOT								
Bled	230 (86)	123	107	1.15	21	(7/12–48)	3	(2 day–10)
Missed	38	18	20	0.90	3	(1/12–48)		N.D.
Combined	268	141	127	1.11	20	(1/12–48)		
Forestry								
Bled	170 (82)	117	53	2.21	22	(9/12–60)	2	(1 day–13)
Missed	37	18	19	0.95	11	(9 day–59)		N.D.
Combined	207	135	72	1.88	21	(9 day–60)		
Highway								
Bled	97 (65)	63	34	1.85	26	(4/12–54)	3	(3 day–43)
Missed	53	24	29	0.83	10	(1/12–75)		N.D.
Combined	150	87	63	1.38	21	(1/12–75)		
All Depts								
Bled	497 (80)	303	194	1.56	22	(4/12–60)	3	(1 day–43)
Missed	128	60	68	0.88	9	(9 day–75)		
Combined	625	363	262	1.39	21	(9 day–75)		

* Time is in years unless otherwise indicated.

Table 2. Frequency of HI Antibody Rise to Arboviruses in 246 Residents of KYNP

Antigen	Four–fold Increase No.	(%)	Eight–fold Increase No.	(%)
Group A				
Chikungunya	6	(2.4)	0	(0.0)
Group B				
T–1674	7	(2.8)	2	(0.8)
Dengue 2	12	(4.9)	4	(1.6)
Japanese Encephalitis	15	(6.1)	7	(2.8)
Any Group B	19*	(7.7)	7	(2.8)

* Group B arbovirus incidence rate = 19/246/yr = 77.2/1000/yr

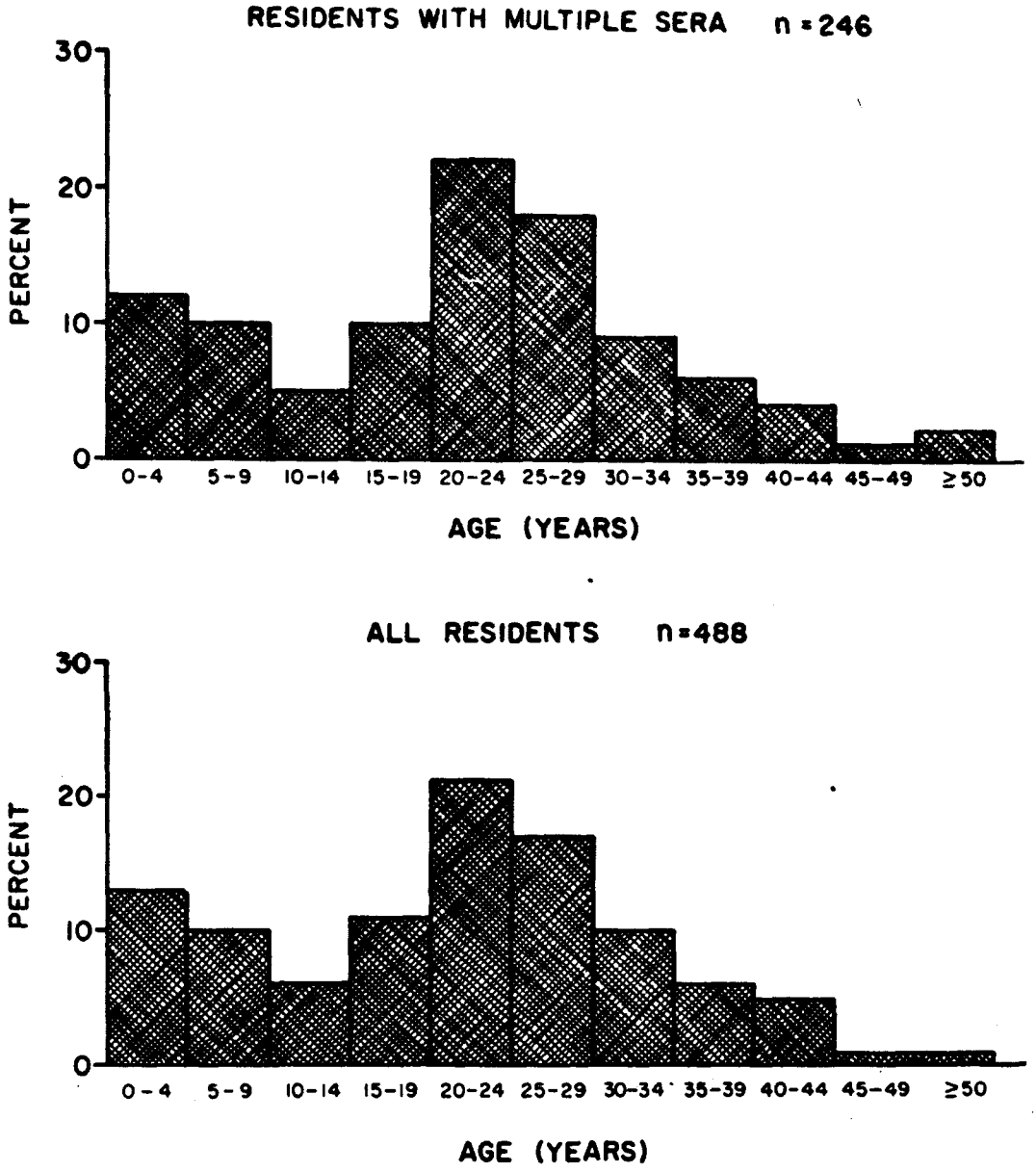

Figure 1. Percent age distribution of residents of Khao Yai National Park

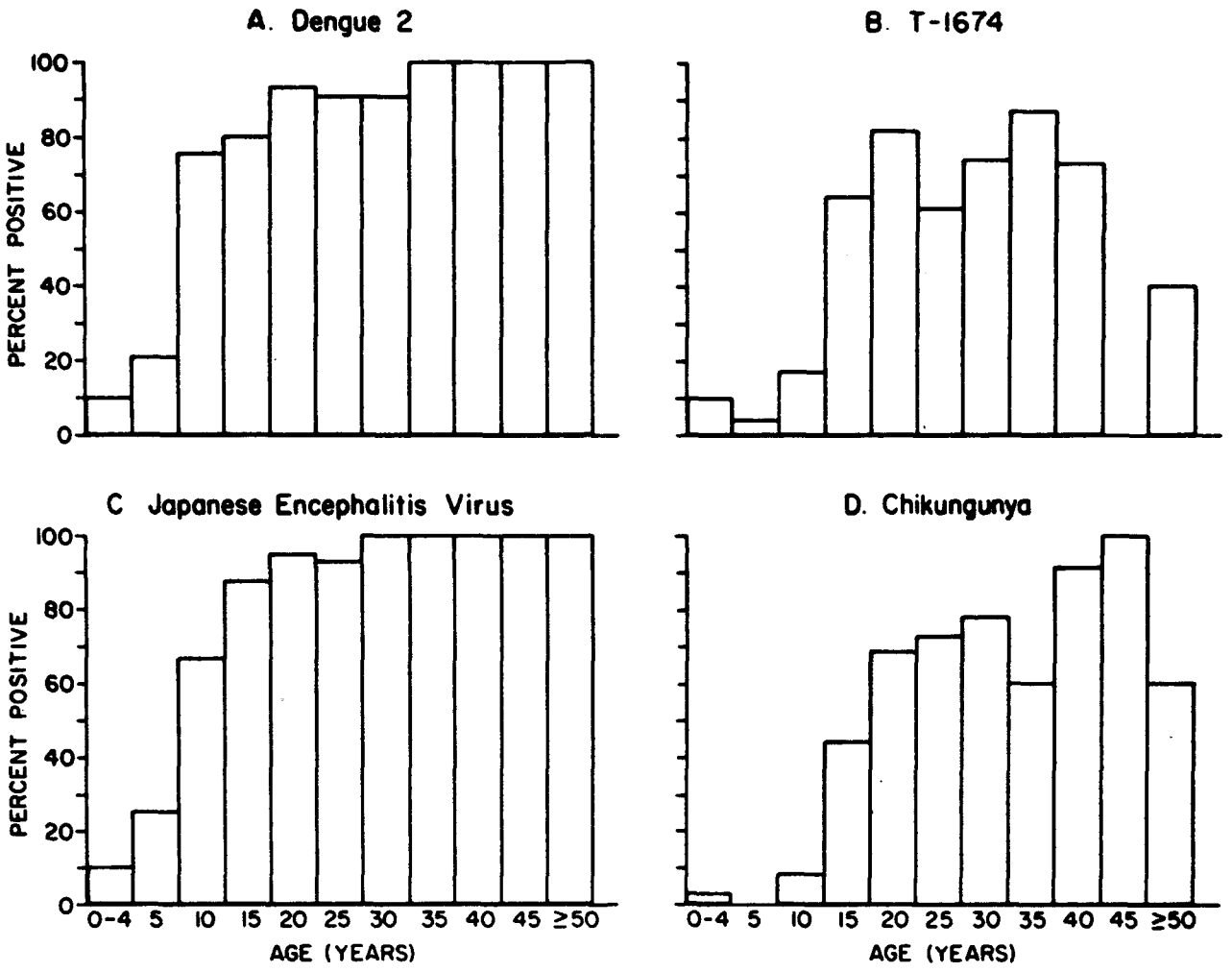

Figure 2 Age specific prevalence of HI antibody to A. Dengue type 2; B. T-1674; C. Japanese encephalitis Virus; and D. Chikungunya in 246 residents of Khao Yai National Park

Epidemic Influenza in A Hill Tribe in Northwest Thailand

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OBJECTIVE: To investigate an epidemic of respiratory disease in the Karen hill tribes of northwest Thailand.

BACKGROUND: The town of Mae Sariang is located on the banks of the Yuam River in a mountainous region of northwestern Thailand (97° 52' longitude, 18° 10' north latitude, at 350 meters above sea level). From Mae Sariang a partially paved road runs north along the river 140 Km to the provincial capital of Mae Hong Sorn. Another road runs 193 Km through the mountains east from Mae Sariang to the city of Chiang Mai (Figure 1 and 2).

The people of this region are largely Karen. They live in small isolated hillside villages of 10—500 houses and are subsistence farmers. Due to the isolation of the villages, travel is largely by foot; it is usually limited to occasional visits to local villages and rarely, in an emergency, to nearby towns. Educational opportunities are rare and there is little understanding of simple health measures. Malnutrition, vitamin deficiency and parasitic infestations are common problems. The climate of this area is influenced by the southern monsoon winds, with the wet season from May to October, and the dry season from November to March. The Christian Medical Unit (CMU) of the American Baptist Misson is located in Mae Sariang. It is a ten—bed hospital with one fulltime physician (BES) and it provides medical service to an estimated 20,000 people who live within a six day walk. Since 1973 the hospital has used a mobile medical unit to make visits every six weeks to hill tribe villages up to three day walk from the road.

In the third week of March 1974, an increase in respiratory disease was reported in Karen villages. The onset of the outbreak was temporally related to a two day meeting of the Karen Baptist Association (KBA) which was attended by an estimated 300 residents of Karen villages. The meeting was held in the village of Mae Hae, located approximtely 38 Km northeast of Mae Sariang, 10 hours on foot from the nearest road (Figure 2). This village is composed of 40-50 houses with an estimated population of approximately 280 residents. At the time of the meeting 60—75 residents (23—27 %) of the village were acutely ill with respiratory symptoms. Many people in surrounding villages also had acute respiratory disease and one village reported seven deaths. Over the two weeks following this meeting 237 patients were seen by the CMU in villages north of Mae Sariang. Many of these were people who had been present at the KBA meeting including one of the CMU staff.

On 5 April the SEATO Medical Research Laboratory was requested by the staff of the CMU to help determine the etilogy of this epidemic.

DESCRIPTION: From 7—9 April 1974 a field team was deployed from the SEATO Medical Research Laboratory to substantiate reports of increased respiratory disease among the Karen people.

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1 Christian Medical Unit, American Baptist Mission, Mae Hong Sorn, Thailand.

Clinical studies: People were examined in villages selected along migration routes so as to monitor past and current disease along these routes. Four villages were selected to the north and three villages to the south of the Mae Sariang – Chiang Mai road (Figure 2). The clinical presentation of the illness was determined by interviewing and examining sick patients with respiratory symptoms. Clinical samples were taken from all people examined and blood was obtained for serology.

Laboratory studies: Throat washes or swabs were obtained on all patients for virus isolation. The techniques for isolation and identification of influenza viruses have appeared elsewhere (1). Briefly, viruses were isolated in embryonated chicken eggs and primary monkey kidney (MK) tissue culture (*Macaca mulatta*). The presence of virus was recognized by hemagglutination or hemadsorption of guinea pig red blood cells. Prototype strains of previously isolated influenza viruses were obtained from Dr. Franklin H. Top, Jr., Walter Reed Army Institute of Research, Washington, D.C. Specific antisera to both the isolates and the prototype strains were prepared in roosters. Isolates were identified by hemagglutination inhibition (HI) using eight hemagglutinating units of antigen and homologous and heterologous rooster antisera. Neutralization tests used 100 $TCID_{50}$ of virus and quantitative neutralization tests were done using 10 fold dilutions of each virus against dilutions of rooster antisera (2).

Blood was obtained for serology on all patients; sera were tested for antibody to influenza by an HI test. Blood smears were obtained on older patients for estimation of the white blood count (WBC) and differential counts. Blood cultures were taken when indicated and throat swabs were obtained from all patients for bacteriological culture.

PROGRESS:

Distribution of cases: Among villages surveyed, disease was evident only in those to the north of the road between Mae Sariang and Chiang Mai. The inhabitants of these villages were largely Christian. Residents of each had attended the KBA meeting and many had developed respiratory disease during the meeting or shortly after returning to their homes. At the time of the survey there was a marked increase in respiratory disease among infants and children; however, respiratory disease among older people was reported to have occurred approximately two weeks earlier. Village headmen estimated that between 10 and 40% of the people in four villages had recently been sick. People in the three villages south of the road were not Christians. No one from these villages had attended the KBA meeting and there was no respiratory disease seen or reported for several months prior to the time of the survey in these villages.

Characteristics of illness: The clinical presentation of the illness was determined by interviewing and examining 25 patients. People of all ages were sick (Table 1). Twelve of the 25 were less than 10 years old and the oldest was 46 years old. Patients seen were said to have been ill from one to 17 days. All had a history of fever and the older ones complained of headache, malaise and prostration. All developed a characteristic hacking cough which in some cases was productive of sputum. The majority had hyperemic throats, and one child had a mild exudative tonsillitis. Eight of the 25 patients had chest findings ranging from scattered ronchi to evidence of consolidation. In 13 patients studied, white blood counts of 10,000 or less were found in nine and differentials showed an absolute lymphocytosis (35–84%) in eight.

Bacterial cultures: Bacterial cultures at blood were obtained from three patients; pneumococcus was recovered from one of these. This individual had first developed illness about one week prior to being seen and had an acute exacerbation of his symptoms eight hours prior to examination. No other bacterial pathogens including beta hemolytic streptococcus were identified in either the blood or the throat cultures.

Virus isolation and identification: Despite difficulties in transportation and storage, nine virus strains were isolated from the pharyngeal secretions of the 25 patients (36%) (Table 1). In one village, isolations were made in six of the eight patients examined. Isolates were easily passed in MK cells or embryonated eggs. No evidence of cytopathogenic effect was noted in the MK cells after as long as 14 days of incubation.

Table 1. Age Distribution of Respiratory Disease and Influenza Isolates of 25 Patients Examined in Northwest Thailand

Age (Years)	Patients Examined	Influenza Isolates
0–9	12	5
10–19	1	0
20–29	3	1
30–39	5	1
≥ 40	4	2
TOTAL	25	9 (36%)

Antisera prepared in roosters against two of the isolates had an HI titer of 1:320 when tested against the homologous antigens and titered within a two-fold dilution when tested against the other strains (Table 2). These results indicate that there were no significant antigenic differences among the isolates from this epidemic.

To determine the extent of the differences in antigenic configuration between the current strain (Mae-Sariang/74) and earlier isolated strains, rooster antisera prepared against the isolates and prototype influenza strains were tested against homologous and heterologous viruses. The HI test demonstrated a close relationship between the current strain and prototype A/Port Chalmers/1/73 (Table 3).

It has been suggested that the neutralization test is more sensitve to antigenic variation than is the HI test (2). When antisera were tested by neutralization, a disparity was revealed in the antibody activity of antisera prepared against these strains. Antisera to A/Port Chalmers/1/73 equally neutralized at high titers both the homologous and the current strains. However, when antisera to the current strains were used, neutralization of 100 $TCID_{50}$ of A/Port Chalmers/1/73 repeatedly required 8-fold more antisera than did the current strain (Table 4). The degree of disparity was not sufficient to differentiate a new influenza strain when analyzed by the method of Archetti and Horsfall (3). These findings, however, do suggest minor antigenic differences between the prototype strain and the present isolate. Quantitative neutralization tests using three viruses against antisera prepared against them substantiated these minor differences (Figures 3 & 4).

HI antibody response: Serum samples were obtained from 25 patients. Unfortunately, due to the remoteness of the area, convalescent samples were not available. In all the individuals from whom virus was isolated the titers were < 1:10. Antibody was present in 14 of the remaining 16 people.

DISCUSSION: That this epidemic was an outbreak of influenza has been amply demonstrated. Influenza virus, closely resembling A/Port Chalmers/1/73 was isolated from 36% (9/25) of the throat secretions collected from acutely ill patients.

The magnitude and extent of the epidemic and the incidence of disease could not be accurately assessed. The population of the hill tribes can only be roughly estimated and the number and distribution of the villages affected is unkown. An incidence of influenza might be inferred from the attack rates reported

for the people of Christian sentinel villages, where 10–15% of the population was said to have been involved.

In Thailand, over the past several years, influenza has usually appeared during September, October or November in Bangkok or at the Royal Thai Air Force Bases. It occurred at a time when resurgence of disease was occurring in other parts of Asia, Europe and North America and was probably introduced into Thailand from these areas. Two epidemics have been studied in the spring when the incidence of influenza was low elsewhere. Both of these were noted first in rural areas; one in Korat in April 1971 and this one in Mae Sariang. We have no information as to the source of this epidemic. The virus may have been introduced into the hills from the central valley of Thailand; a mild outbreak of influenza occurred in Bangkok in October and November of 1973, from which a virus similar to the A/Port Chalmers /1/73 strain was isolated. Alternately, the virus may have spread south through the hills from Burma, Laos or China. Consistent with this hypothesis is the occurrence of respiratory illness in hill tribe villages near Fang, 200 Km to the north of Mae Sariang in February 1974 (personal communication: Prince Pisadej Rachanee, Director, His Majesty's Hill Tribe Project, Chiang Mai, Thailand).

Influenza may have resulted in a recognizable epidemic through a series of unusual and fortuitous circumstances. Rare in itself was the gathering of individuals from many villages at the KBA meeting.

Table 2. Hemagglutination Inhibition Test on Nine Virus Strain from Isolated Patients in Mae Sariang Using Rooster Antisera Prepared to Two of Them

Antiserum / Antigen	Reciprocal Hemagglutination Titers	
	SM/898/74	MS/913/74
MS/862/74	320	320
MS/868/74	320	320
MS/871/74	320	320
MS/872/74	320	320
MS/874/74	320	320
MS/877/74	320	320
MS/878/74	320	320
MS/883/74	320	320
MS/886/74	320	320
MS/913/74	320	320
MS/916/74	640	640

Table 3. Comparison by Cross Hemagglutination Inhibition of Current Influenza Strains with Prototype Strains of Previously Isolated Influenza Viruses

Antisera[a] / Antigen[b]	Reciprocal Hemagglutination Inhibition Titers					
	A/MS/868/74	A/P.Chal/1/73	A/Eng/42/72	A/H.K./1/68	A/Jap/305/57	B/Lee/40
A/MS/868/74	320	320	160	80	<10	<10
A/P.Chal/1/73	320	320	320	160	<10	<10
A/Eng/42/72	80	160	160	160	<10	<10
A/H.K./1/68	80	80	80	320	10	<10
A/Jap/305/57	40	20	80	80	160	<10
B/Lee/40	<10	<10	<10	<10	<10	1280

a Specific rooster antisera

b Hemagglutination inhibition test used 8 hemagglutinating units.

Table 4. Comparison by Cross Neutralization of Current Influenza Strains with A Influenza/H_3N_2/ viruses

Antisera[a] / Antigen[b]	Reciprocal of Neutralizing Antibody Titer			
	A/MS/868/74	A/P. Chal/1/73	A/Eng/42/72	A/H.K./1/68
A/MS/868/72	160	160	20	20
A/P.Chal/1/73	20	160	20	20
A/Eng/42/72	20	80	160	20
A/H.K./1/68	20	40	40	160

[a] Specific rooster antisera

[b] Neutralization tests used 100 $TCID_{50}$ of the appropriate virus.

The almost exclusive involvement of Christian villages, as opposed to non—Christian villages, implicate this meeting as a point source for the local epidemic. This led to the infection of people from widely scattered villages and ultimately to a simultaneous increase in disease over a large area. The epidemic probably would not have been recognized were it not for the activities of the CMU mobile unit with its program of medical service to isolated villages.

The data collection for this study is complete. We are awaiting the final identification of strains of influenza virus isolated in Bangkok during the summers of 1973 and 1974. Upon receipt of this information this work will be analysed and prepared for publication.

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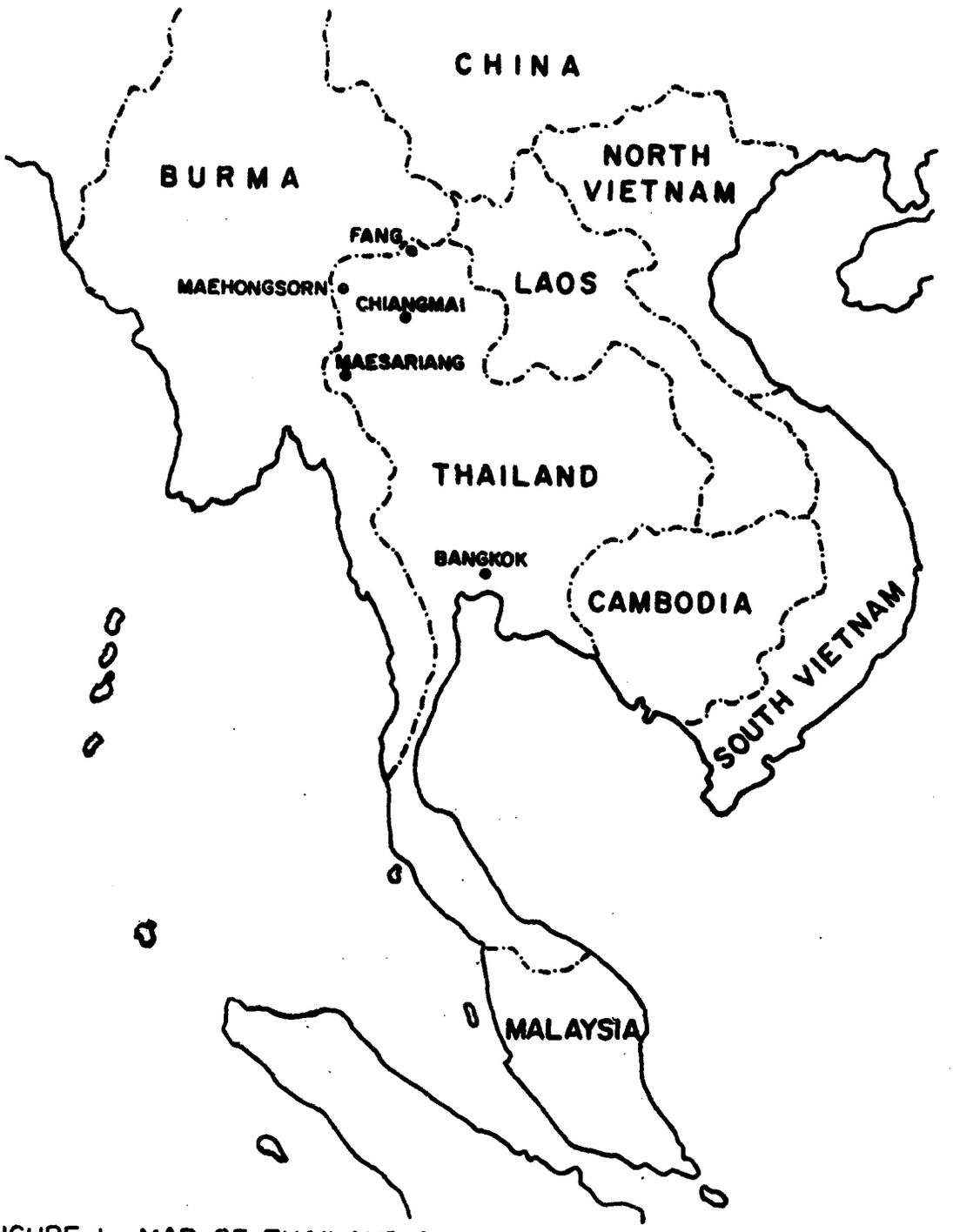

FIGURE 1 MAP OF THAILAND SHOWING THE TOWN OF MAE SARIANG IN RELATION TO CHIENG MAI, MAE HONG SORN, FANG AND BANGKOK.

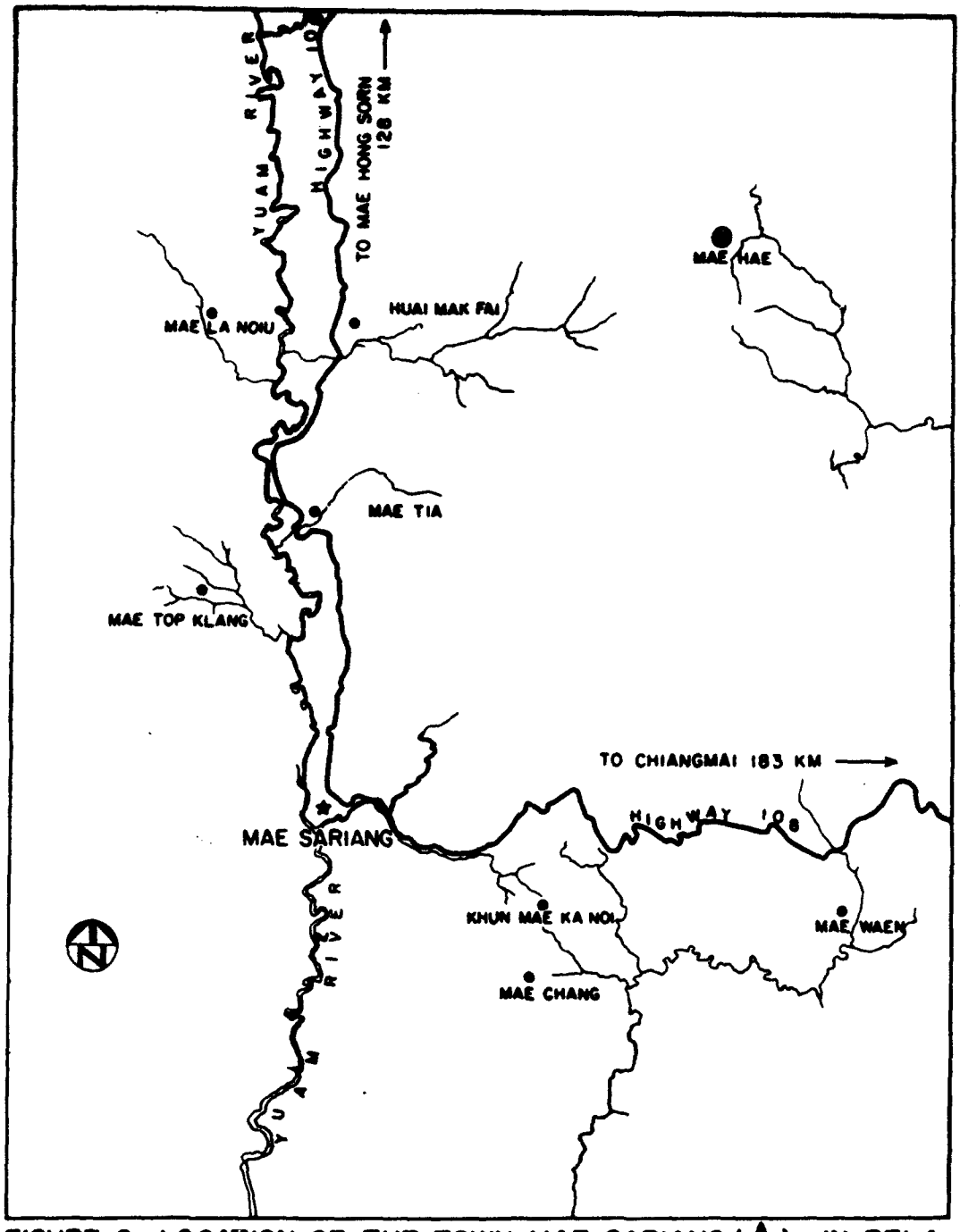

FIGURE 2. LOCATION OF THE TOWN MAE SARIANG (★), IN RELATIONSHIP TO THE YUAM RIVER (═), THE HIGHWAY (▬), THE SENTINEL VILLAGES (●) AND THE SITE OF THE MEETING OF THE KAREN BAPTIST ASSOCIATION AT BAN MAE HAE (●).

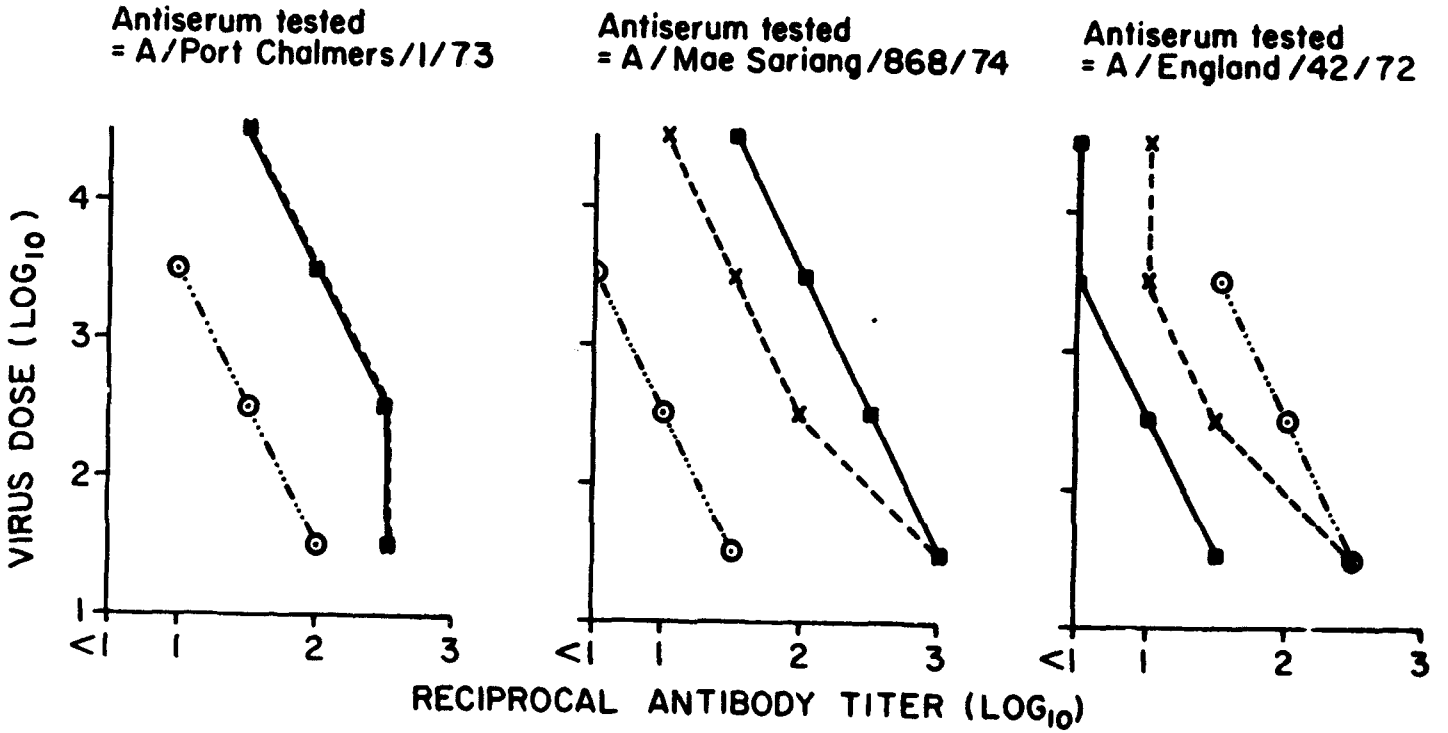

Figure 3. Quantitative relationships between recently isolated A/Influenza/H_3N_2/strains. Comparisons of neutralization reactions of A/Port Chalmers/1/73, A/Mae Sariang/868/74 and A/England/42/72 showing dissimilar patterns of neutralization. Viruses tested x----x A/Port Chalmers/1/73, ■——■ A/Mae Sariang/868/74, ⊙-·-·-⊙ A/England/42/72

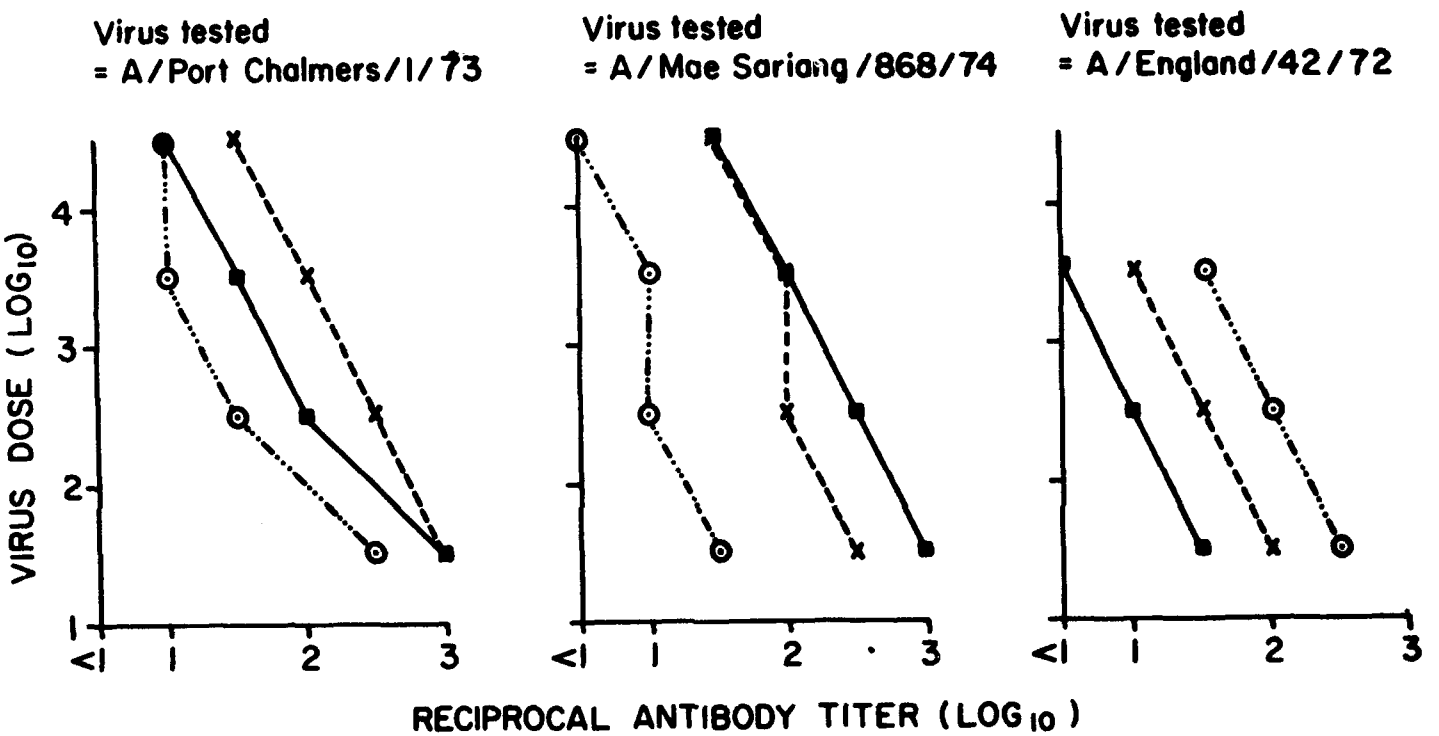

Figure 4. Quantitative relationships between recently isolated A/Influenza/H_3N_2/ strains. Comparisons of neutralization reactions of A/Port Chalmers/1/73, A/Mae Sariang/868/74 and A/England/42/72 showing dissimilar patterns of neutralization. Antisera tested x----xA/Port Chalmers/1/73, ●——● A/Mae Sariang/868/74, ⊙-·-·-·⊙ A/England/42/72

Rabies Exposure During Pregnancy

Principal Investigators: Michael R. Spence, MAJ, MC
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Associate Investigator: Prakorb Boonthai, M.D.[1]

OBJECTIVE: To study transfer of rabies virus and antibody across the human placenta.

BACKGROUND: Rabies virus has been shown to cross the placenta in experimental infections in many species (1 – 3). Transplacental transmission of this virus has also been reported following a naturally acquired infection in a pregnant cow (4). This phenomenon has not been reported to occur in man. We have recently seen two patients with rabies exposure during the third trimester of pregnancy.

PROGRESS: The first patient, a 43 year old Thai female, was approximately eight months pregnant when she was bitten on the leg by a stray dog. The patient cleansed the wound with soap and water and sought medical advice. No specific antirabies therapy was initiated, presumably because she was pregnant.

On 6 December 1972 the patient delivered a healthy male infant, without complications. On 8 December 1972, the patient's second postpartum day, she developed symptoms of encephalitis. Her condition progressively deteriorated and she died on 12 December 1972. Corneal impressions and a blood sample were obtained two hours prior to her death. An autopsy was performed. The corneal impressions and tissues obtained at autopsy were fixed and stained with fluorescein labelled anti–rabies globulin (5). The fluorescent antibody stained antemortem and postmortem corneal impressions were positive, while skin taken from the wound site was negative. Positive results were also obtained from the hippocampus, cerebrum, cornea and lacrimal gland. The serum sample contained no neutralizing antibodies to rabies (Titer < 1:5) by mouse serum neutralizing antibody test. Serum specimens from the infant were also negative for rabies neutralizing antibodies in the neonatal period and again one year later. The child is alive and well at two years of age.

Case 2 was a 29 year old Caucasian female. At 35 weeks gestation the patient was bitten on the hand and arm by a pet cat subsequently found to be rabid in our laboratory. The wounds were cleansed with soap and water and 4000 U of antirabies hyperimmune serum was administered intramuscularly. Immunization with killed rabies virus vaccine (Duck Embryo Origin) was initiated. The immunization schedule was single, daily injections for fourteen days followed by three boosters on days 24, 34, and 64. On 18 April 1974, at 39 weeks gestation, the patient's labor was induced and she delivered a healthy male infant without difficulty. A maternal serum sample was obtained prior to induction, and cord blood was collected at delivery. Serum samples were also obtained from the patient and her infant at three and six weeks postpartum. All serum samples were evaluated for the presence of rabies neutralizing antibodies. The results of these tests are indicated in Table 1. The patient received a booster immunization at four weeks postpartum. Both the patient and her infant are alive and well nine months after delivery.

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Table 1. Serum Rabies Neutralizing Antibody Titers Obtained from Patient 2 and her Infant

SAMPLE	TITER
Preinduction	
Maternal blood	1 : 70
Cord blood	1 : 30
Three Weeks postpartum	
Maternal blood	1 : 40
Infant blood	1 : 5
Six weeks postpartum	
Maternal blood	1 : 80
Infant blood	< 1 : 5

DISCUSSION: The infant of the first patient demonstrated no evidence of acquiring his mother's infection. Additionally he had no detectable immune response to rabies virus. These findings suggest that the virus did not cross the placenta.

The second patient demonstrated a good immunologic response to antiserum and vaccine. The presence of antibodies in her infant's serum that rapidly decreased in titer with time suggests that these antibodies crossed the placenta resulting in passive immunization. Antibody titers in maternal blood at the time of induction of labor may be different from the titers in the cord blood because maternal IgM antibody did not cross the placenta.

It is not our opinion that, in our single case report, we have established the safety of antirabies immunization during pregnancy. We do feel, however, that pregnancy is no contraindication to rabies immunization.

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Animal Rabies in Thailand: Rabies Diagnostic Laboratory Services

Principal Investigators: Garrett S. Dill, Jr., CPT, VC
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OBJECTIVE: To provide rabies diagnostic services to U.S. military personnel in Southeast Asia and the Western Pacific.

DESCRIPTION: Every brain submitted to this laboratory was examined by both the fluorescent antibody test and by mouse inoculation. Agreement between the two tests was 99.87%.

PROGRESS: Of 758 brain specimens examined, 253 (33.4%) were positive (Table 1). The prevalence of rabies in the dog (42.2%) and in the cat (11.5%) was slightly less than in recent years.

Table 1. Summary of Rabies Diagnoses—1 April 1974—31 March 1975

Species	Number of Specimens	Number Positive	Percent Positive
Canine	555	234	42.2
Feline	122	14	11.5
Human	2	2	100.0
Rodent	24	2 (a)	8.3
Primate	21	1 (b)	4.8
Rabbit	11	0	0
Bat	10	0	0
Squirrel	5	0	0
Other (c)	8	0	0
Total	758	253	33.4

(a) Two rats from the Republic of the Philippines
(b) Pet gibbon from Nakorn Panom
(3) 3 civets, 1 goat, 1 mole, and 3 guinea pigs

Prevalence of Some Viral Infections in the Residents of Phnom – Penh

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Panor Srisongkram, B. Sc.
Nonglak Kananulaksa, B. Sc.

OBJECTIVE: To survey the experience of residents of Phnom – Penh with polioviruses, mosquito – borne arboviruses and hepatitis B virus (HBV).

BACKGROUND: The question of transmission of hepatitis B virus remains an enigma. It has been shown that virus may be passed by the parenteral route, and contaminated needles or parenteral preparations were implicated in the transmission of disease. Recently the question of arthropod transmission has been raised as a mode of transmission in the tropics. Non – parenteral transmission has also been demonstrated experimentally. Epidemiological investigations have implicated this mode of transmission in tropical areas.

Sera from a Cambodian population were collected by the National Blood Transfusion Center and the Institute of Biology, Phnom – Penh, in a pre – polio immunization study to determine the optimum age of polio immunization in this population. This population resided in a tropical area with generally poor sanitation, and no known recent polio immunization. Biting arthropods are prevalent in Phnom – Penh and many of these are known to be virus vectors. Prior studies had indicated that this population should have a high prevalence of hepatitis B surface antigen (HB_sAg) carriers (1). This information suggested that a comparison of the experience with polio, arboviruses and hepatitis B infections in this population might shed some light on the mode of transmission of HBV in the tropics.

DESCRIPTION: This study was done in collaboration with the National Blood Transfusion Center and the Institute of Biology, Phnom – Penh at the request of Dr. Rene Sansonnens, a WHO representative. Questionnaires were completed by 370 Cambodians presenting themselves for immunization against polio. Sera were obtained from all 370 people prior to immunization. Sera were screened at a dilution of 1:10 for antibody against polio types one, two and three using a metabolic inhibition technique with LLC – MK_2 cells (2). Antibody to mosquito – borne arboviruses found in Southeast Asia were detected by a hemagglutination inhibition test (2) using Chikungunya (Ross) virus as representative of group A arboviruses, Dengue 2 (New Guinea) and Japanese Encephalitis (Nakayama) viruses as representative of group B arboviruses. Sera with no antibody detectable at a 1:10 dilution were considered to be negative. Hepatitis B surface antigen (HB_sAg) was detected by immunoelectrophoresis (IEOP) using high titered human antiserum. The technique of this test has been previously described (3).

Antibody to hepatitis B surface antigen will be detected by either a radioimmune assay inhibition (RIAI) or a passive hemagglutination test (PHA, Electronucleonics).

PROGRESS: Polio antibody was present in approximately 25% of children under one year of age (Table 1). Prevalence of antibody increased rapidly, approached 100% by the age of four years and remained between 90 and 100% in all older age groups studied. At least 50% of the children between the age of six to nine years had acquired antibody to all three types of polioviruses. By the age of 15, approximately 85% of the population had antibody to type one, 92% to type two and 28% to type three. These data indicate a high incidence of orally transmitted polio infection in this population over the first few years of life.

Almost 50% of children under the age of one year had experience with group B arbovirus infections (Table 2). This figure dropped significantly in the second year of life to 38% but rose rapidly approaching 100% by the fourth year of life. Antibody to group A arboviruses rose more slowly, being present in 26% of children studied under one year of age. As with group B arboviruses, antibody prevalences fell during the second year. After that, group A antibodies rose more slowly than group B, approaching 100% by 13 years of age.

Twenty-nine individuals or 8% of this population were found to be HB_s antigenemic at the time of collection (Table 3). HB_sAg was found in 4.6% of children under four years of age and rose to about 19% by the age of 15-19. The age specific point prevalence of anti-HB_s remains to be determined.

DISCUSSION: A conclusion from this work is that vaccination against poliomyelitis in this population is indicated for children under the age of four years.

These data indicate extremely rapid transmission of both polio (acquired by the oral route) and group A and B arboviruses (acquired by the parenteral route). HB_sAg appeared in very young children but it reached a peak prevalence later than that of polio or arbovirus antibody (Figure 1).

The prevalence of antibody to HB_sAg has not yet been determined. It remains to be seen whether the transmission rate of HBV is similar to that of polio or arboviruses or occurs at a slower rate. Experience in Bangkok, with a lower socio-economic housing development suggests that the latter might be the case.

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Table 1. Age Specific Point Prevalence of Polio Neutralizing Antibody in Residents of Phnom-Penh

Age (years)	No. Tested	Negative all 3 types		Positive Ab all 3 types		Positive Neutralizing Antibody to					
						Polio 1		Polio 2		Polio 3	
		No.	%	No.	%	No.	%	No.	%	No.	%
0–1	20	15	75.00	2	10.00	3	15.00	3	15.00	3	15.00
1–2	29	12	41.38	1	3.45	10	34.48	6	20.69	11	37.93
2–3	15	1	6.67	2	13.33	8	53.33	4	26.67	10	66.67
3–4	22	7	31.82	4	18.18	11	50.00	10	45.45	8	36.36
4–5	25	1	4.00	2	8.00	14	56.00	17	68.00	12	48.00
5–6	36	1	2.78	9	25.00	26	72.22	29	80.56	20	55.56
6–9	36	2	5.56	18	50.00	23	63.88	31	86.11	25	69.44
9–12	21	1	4.76	13	61.90	15	71.42	18	85.71	17	80.95
12–15	40	0	0	26	65.00	34	85.00	37	92.50	33	82.50
15–20	30	1	3.33	16	53.33	23	76.67	28	93.33	20	66.67
20–25	50	2	4.00	26	52.00	36	72.00	45	90.00	32	64.00
25–30	30	3	10.00	15	50.00	23	76.67	26	86.67	16	53.33
30–35	15	1	6.67	7	46.67	12	80.00	14	93.33	8	53.33
35–40	1	0	0	1	100.00	1	100.00	1	100.00	1	100.00

Tabe 2. Age Specific Point Prevalence of HI Antibody to Mosquito-borne Arboviruses in Residents of Phnom-Penh

Age (years)	No. Tested	No Ab to both Gr. A and Gr. B		Positive Ab to both Gr. A and Gr. B		Positive Ab to Gr. A		Positive Ab to. Gr. B.	
		No.	%	No.	%	No.	%	No.	%
0–1	19	8	42.18	3	15.79	5	26.50	9	47.36
1–2	29	18	62.00	5	17.24	5	17.24	11	37.93
2–3	15	4	26.70	3	20.00	3	20.00	11	73.33
3–4	23	1	4.34	6	26.08	7	30.43	21	91.30
4–5	25	3	12.00	7	28.00	7	28.00	22	88.00
5–6	35	4	11.42	14	40.00	15	42.85	30	85.71
6–9	36	2	5.56	19	52·78	19	52.78	34	94.44
9–12	21	1	4.76	13	61.90	13	61.90	20	95.24
12–15	39	0	0	36	92.30	36	92.30	39	100.00
15–20	29	0	0	24	82.75	24	82.75	29	100.00
20–25	50	0	0	49	98.00	49	98.00	50	100.00
25–30	30	0	0	30	100.00	30	100.00	30	100.00
30–35	15	0	0	14	93.33	14	93.33	15	100.00
35+	1	0	0	1	100.00	1	100.00	1	100.00

Table 3. Age Specific Point Prevalence of HB_sAg in Residents of Phnom-Penh

Ag (years)	Male			Female			Total		
	No. Tested	HBsAg+ve		No. Tested	HBsAg+ve		No. Tested	HA_sAg+ve	
		No.	%		No.	%		No.	%
0–4	55	4	(7.3)	53	1	(1.9)	108	5	(46.)
5–9	35	2	(5.7)	46	3	(6.5)	81	5	(6.2)
10–14	20	4	(20.0)	31	4	(12.9)	51	8	(15.7)
15–19	9	2	(22.2)	23	4	(17.4)	32	6	(18.6)
20–29	3	1	(33.3)	68	4	(5.9)	71	5	(7.0)
30–39	2	0	(0)	16	0	(0)	18	0	(0)
Total	124	13	(10.5)	237	16	(6.8)	361	29	(8.0)

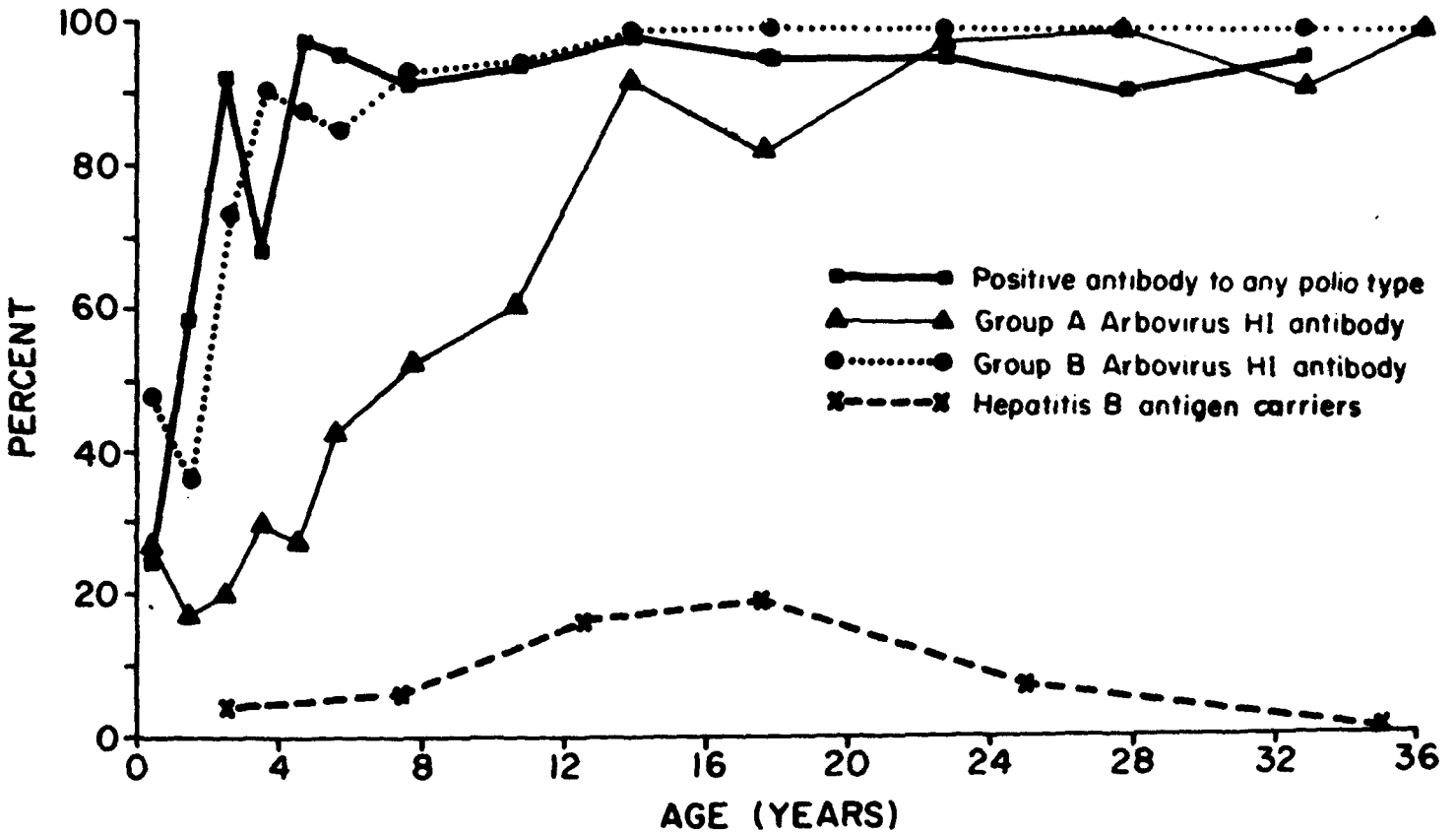

FIGURE 1 AGE SPECIFIC POINT PREVALENCE OF HI MOSQUITO-BORNE ARBOVIRUSES, POLIO NEUTRALIZING ANTIBODIES AND HEPATITIS B ANTIGEN CARRIERS IN RESIDENTS OF PHNOM-PENH (JUNE 1973)

Psittacosis in Birds and Man

Principal Investigators: Vichai Sangkasuwan, LTC, MC, RTA
Manit Kanvallee, Fly. Off., VC, RTAF
Krasare Nabnean, LT, MSC, RTA

OBJECTIVE: To survey the prevalence of psittacosis in birds and man.

BACKGROUND: No report on psittacosis has been made in Thailand, although parakeets and other birds are common pets in this country.

DESCRIPTION: Dead birds were obtained from a local animal and bird dealer. Their blood was collected for a complement fixation (CF) test and the suspension of liver and spleen was inoculated into mice and guinea pigs in an attempt to isolate the psittacosis agent.

Paired sera were collected from patients suffering from pneumonitis and examined for the presence of psittacosis CF antibody.

PROGRESS: The results of isolation attempts are shown in Table 1.

Table 1. Result of the Isolation Attempts for Bedsonia

Month, Year	No. of Birds Examined	No. Positive
Nov 74	152	0
Dec	503	0
Jan 75	303	0
Feb	406	0

DISCUSSION: Twenty—eight species of birds including parakeets were obtained from the local animal and bird dealer. Unfortunately the birds often died at night and were left at ambient temperature and the examination was not made until the following morning. This, perhaps, contributed to the difficulty in isolating the agent.

BACTERIAL DISEASES OF MAN AND ANIMALS

Changing Penicillin Resistance of the Gonococcus in Thailand

Principal Investigators: Michael R. Spence, MAJ, MC
Douglas R. Stutz, MAJ, MSC

Associate Investigators: Chanpen Srimunta, B.S.
Chiraphun Duangmani, M.D.

OBJECTIVE: To determine if the penicillin resistance of the gonococcus in Thailand is changing.

BACKGROUND: Although the relative resistance of the gonococcus to penicillin has been shown to be decreasing in two geographical locales (1,2), most reports indicate that the organism is becoming more resistant at a very alarming rate (3, 4).

DESCRIPTION: Test organisms consisted of all clinical isolates of *N. gonorrhoeae* submitted for confirmation to the Department of Microbiology, SEATO Medical Research Laboratory, Bangkok, Thailand between 1 January 1972 and 31 December 1974. Specimens were clinical isolates submitted from clinical facilities in Thailand. These facilities included: Royal Thai Army Hospital, Bangkok; US Army Hospital, Bangkok; and SEATO Medical Research Laboratory venereal disease field unit. The number of isolates evaluated was: 752 in 1972; 622 in 1973; and 867 in 1974.

All organisms were confirmed to be *N. gonorrhoeae* by standard techniques previously reported. Organisms confirmed as *N. gonorrhoeae* were grown on PB plates (Proteose #3 agar + 1% Hemoglobin + 1% IsoVitaleX*) overnight. The colonies were scraped off with a sterilized swab and heavy suspensions were prepared in tryptose phosphate broth. The suspensinos were standardized spectrophotometrically (Coleman Jr. II, Spectrophotometer, Coleman Instruments Maywood, Ill.) with a $BaSO_4$ solution (0.5 ml 1% $BaCl_2$ + 99.5 ml 1% H_2SO_4) to a final bacterial count of 10^7 colony forming units/ml. Inoculum–replicating equipment (Div. of Instrumentation, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.) placed 0.001–0.003 ml of inoculum in a spot on the surface of a plate containing Mueller–Hinton Agar + 5% defibrinated, chocolatized sheep blood and added 1% IsoVitaleX. Serial dilutions of penicillin G were added to the plates for penicillin minimum inhibitory concentration (MIC) determinations. The concentrations tested were: 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 units/ml. One additional plate to which no penicillin was added was also evaluated. A Staphylococcus strain, *S. aureus* ATTCC 6538 P was used as a control for the penicillin content of the plates. The plates were stored at 4°C after preparation and were employed within three days. Before use the plates were dried of excess moisture in the incubator. No moisture droplets were on the surface of the media or petri dish lids when inoculations were made. The inoculated plates were evaluated after overnight incubation (20–24 hrs.). The MIC was determined as the smallest concentration of antibiotic required to inhibit growth completely. In reading the plates, a barely visible haze of possible growth or a single macroscopic colony was disregarded.

Three study populations consisting of all isolates submitted within a given calendar year were compared. In order to apply statistical methods in evaluating the data all isolates with MIC's of less than 0.5 units/ml or more than 2.0 units/ml were excluded. Thus the MIC data for 14 organisms in 1972, 27 in 1973 and 130 in 1974 were not used in calculating means, standard errors and significance of differences. The MIC results of 362 organisms collected between April 1972 and January 1973 have been discussed elsewhere (5). Analysis of variance was utilized to determine if between–group differences existed (6). Student's t test for unpaired variates was employed to determine the significance of differences between the mean MIC's of the organisms tested within a given year (6).

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*Baltimore Biological, Cockeysville, Maryland 21030, U.S.A.

PROGRESS: The mean penicillin minimum inhibitory concentration ± 2 standard errors for gonococci isolated in 1972, 1973 and 1974 were 0.58 ± 0.02, 0.72 ± 0.04 and 1.05 ± 0.04, respectively. Analysis of variance for between-group differences of these three populations was significant ($F = 215.5$, $df = 2067$, $p < 0.001$).

MIC data obtained in 1972 was significantly different from that of 1973 ($p < 0.001$). An identical result was found when the 1973 data was compared to 1974 ($p < 0.001$).

The data are graphically represented in Figures 1, 2 and 3. Each year the proportion of isolates with a penicillin MIC of more than 2.0 units/ml increased. The mean MIC ± 2 S.E.'s for each of the three years is displayed in Figure 4. Calculations of means and standard errors in Figure 4 were performed excluding the data extremes as explained above.

SUMMARY: Penicillin minimum inhibitory concentrations (MIC) were determined for 2241 gonococcal isolates submitted to the SEATO Medical Research Laboratory between 1 January 1972 and 31 December 1974. Isolates were separated into three groups, determined by the calendar year in which they were submitted. The mean penicillin MIC ± 2 standard errors was calculated for each of the three groups. These values were 0.58 ± 0.02 in 1972, 0.72 ± 0.04 in 1973, and 1.05 ± 0.04 in 1974. These means were found to be significantly different ($p < 0.001$).

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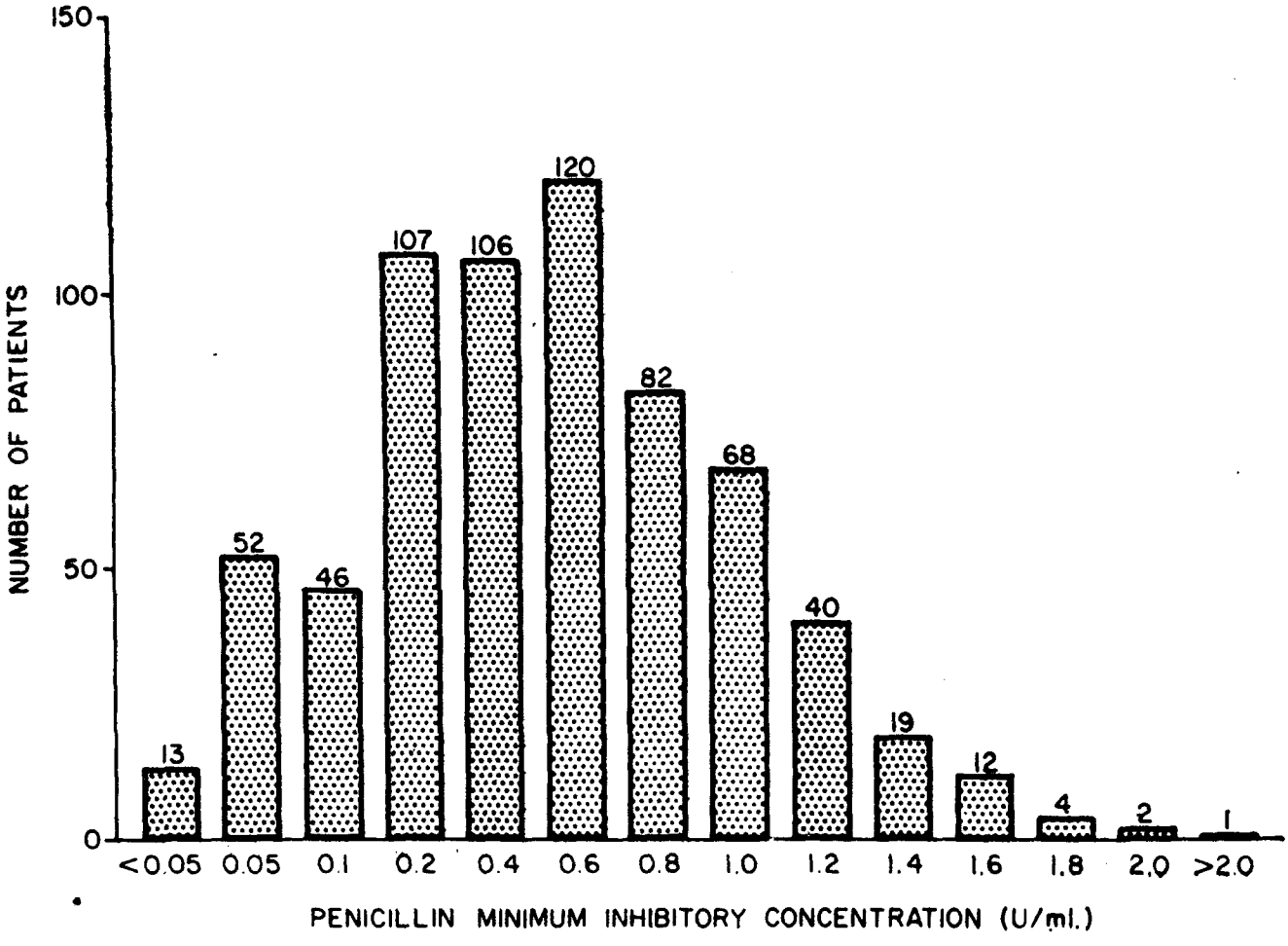

FIGURE 1. PENICILLIN MINIMUM INHIBITORY CONCENTRATION FOR NEISSERIA GONORRHOEAE ISOLATED IN 1972. DATA EXTREMES (0.05 U/ml. >MIC >2.0 U/ml.) WERE NOT INCLUDED IN CALCULATIONS OF MEAN AND STANDARD ERROR

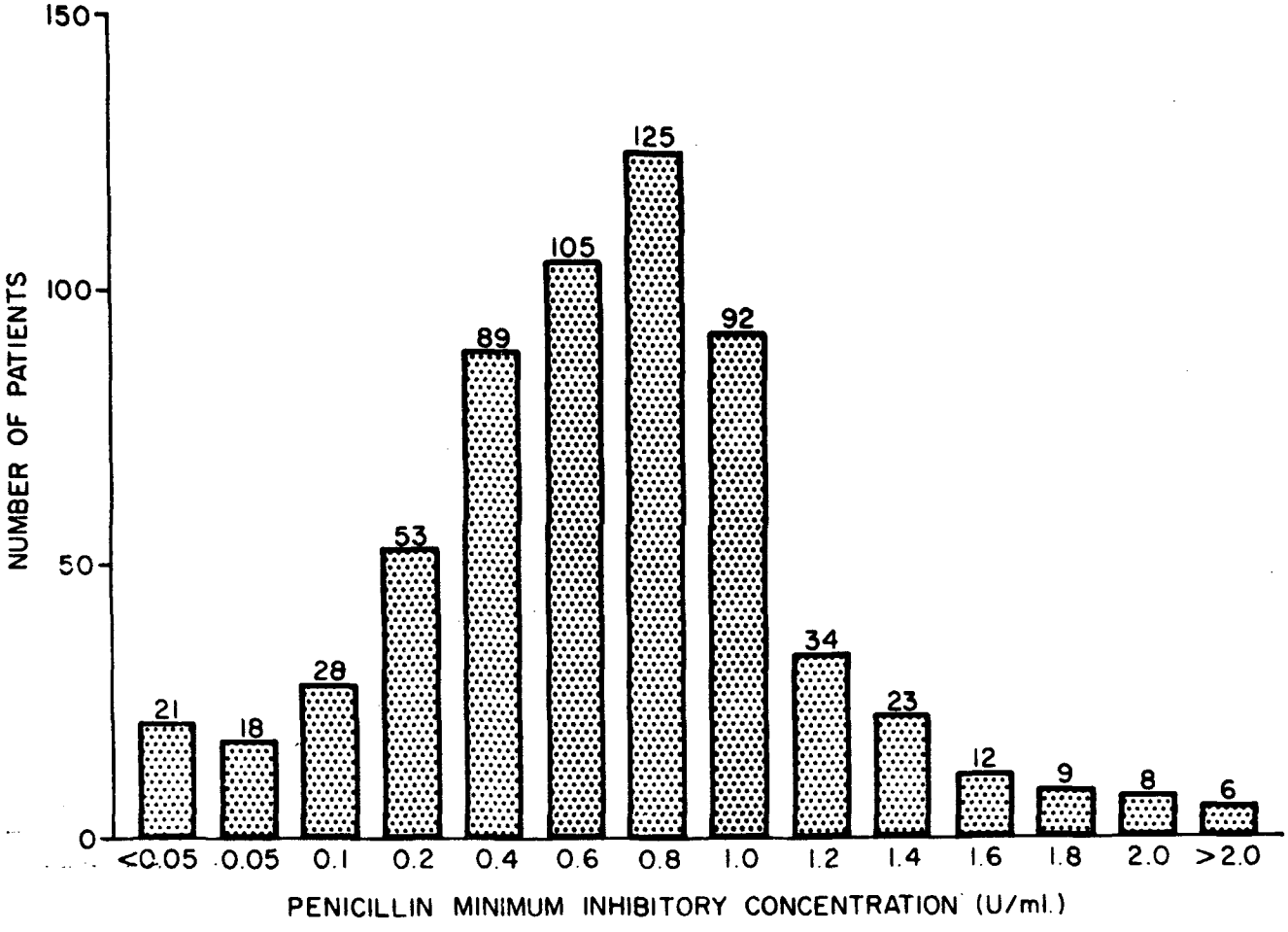

FIGURE 2. PENICILLIN MINIMUM INHIBITORY CONCENTRATION FOR NEISSERIA GONORRHOEAE ISOLATED IN 1973. DATA EXTREMES (0.05 U/ml. >MIC >2.0 U/ml.) WERE NOT INCLUDED IN CALCULATIONS OF MEAN AND STANDARD ERROR

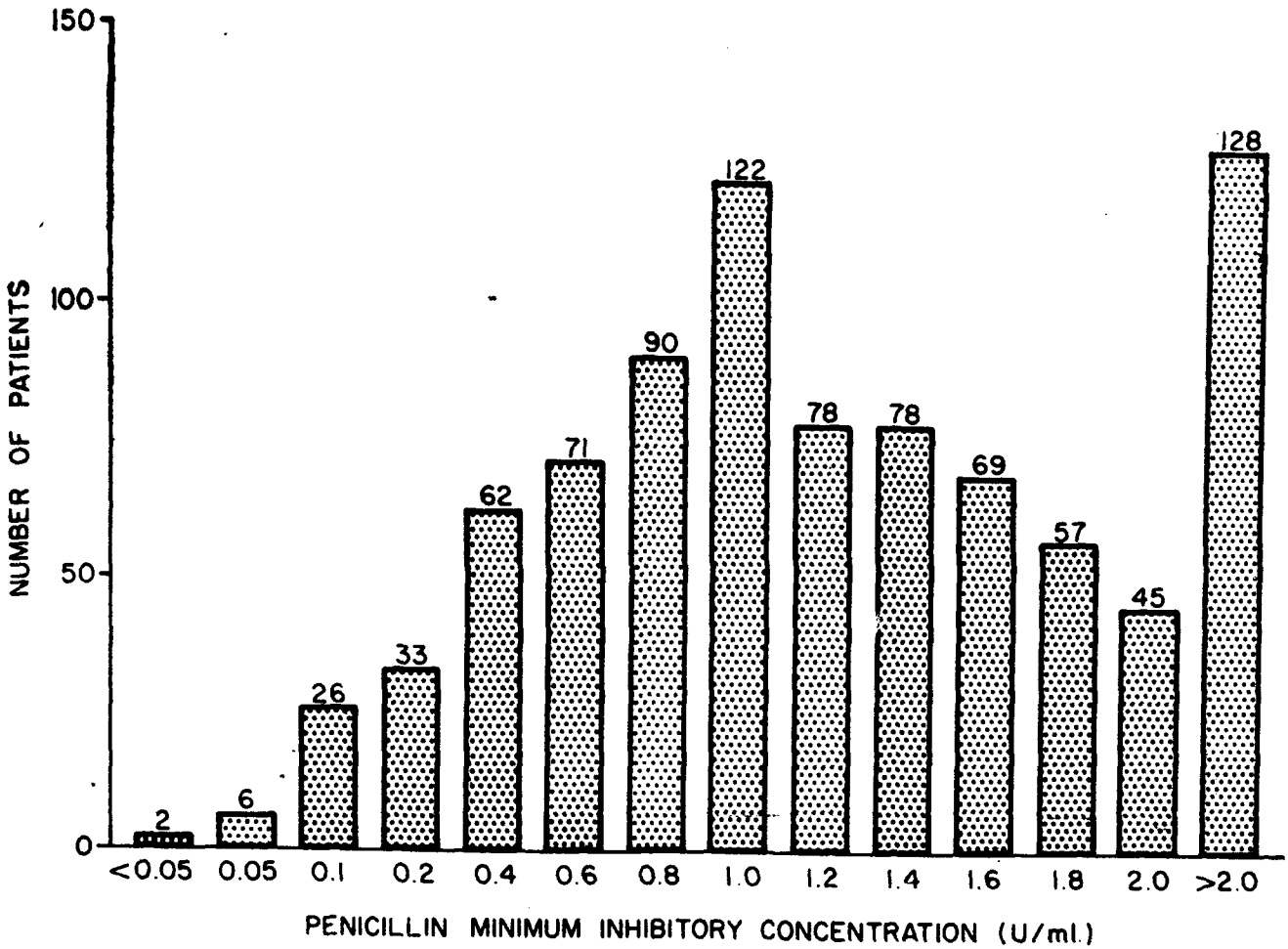

FIGURE 3. PENICILLIN MINIMUM INHIBITORY CONCENTRATION FOR NEISSERIA GONORRHOEAE ISOLATED IN 1974. DATA EXTREMES (0.05 U/ml. >MIC >2.0 U/ml.) WERE NOT INCLUDED IN CALCULATIONS OF MEAN AND STANDARD ERROR

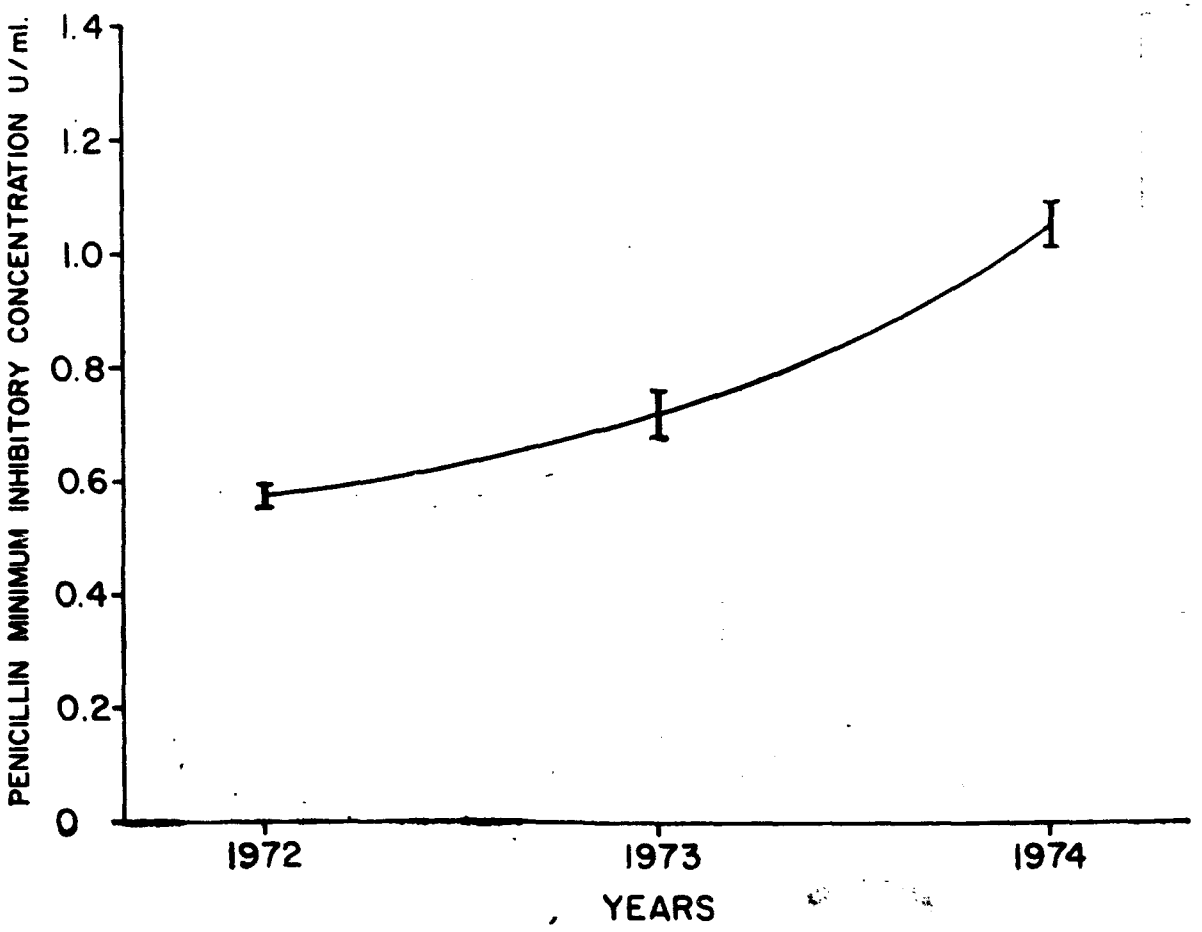

FIGURE 4. MEAN PENICILLIN MINIMUM INHIBITORY CONCENTRATION ± 2 S.E. FOR NEISSERIA GONORRHOEAE ISOLATED IN 1972, 1973 AND 1974. DATA EXTREMES (0.05U/ml. >MIC >2.0U/ml.) WERE DELETED IN MEAN AND STANDARD ERROR CALCULATIONS

Oropharyngeal Gonorrhea During Pregnancy

Principal Investigators: Douglas R. Stutz, MAJ, MSC
Michael R. Spence, MAJ, MC

Associate Investigator: Chiraphun Duangmani, M.D.

OBJECTIVE: To compare the prevalence of *Neisseria gonorrhoeae* infections in a prenatal population of U. S. military dependents to a prenatal population of Thai civilian and a non–pregnant population of female military dependents.

BACKGROUND: *Neisseria gonorrhoeae* was isolated in 16% of 150 pregnant U.S. military dependents (1). Of the 24 positive cultures 23 were obtained from the oropharynx. In view of this finding it was felt that similar studies of Thai prenatal patients and non–pregnant U.S. military dependents should be performed.

DESCRIPTION: Identical techniques to those previously described (1) were employed to obtain specimens from prenatal patients attending the Obstetrics Outpatient Clinic of Women's Hospital, Bangkok, Thailand during the first three months of 1974. A culture of the oropharynx was obtained from all females between the ages of 15 and 24 years who attended the U. S. Army Dental Clinic, Bangkok from 1 January 1974 to 30 March 1974. *N. gonorrhoeae* was isolated and confirmed by methods discussed previously (1).

PROGRESS: Positive cultures were obtained in 19 of 160 (11.9%) Thai patients attending Women's Hospital. No positive cultures were obtained from the oropharynx in this group. Two of 114 patients (1.8%) attending the Dental Clinic had the gonococcus cultured from their oropharynx.

DISCUSSION: No oropharyngeal infections were found in the patients attending the prenatal clinic of Women's Hospital. These patients were predominately housewives and did not admit to the practice of fellatio. The practice of fellatio is not generally accepted in the culture of Thailand; therefore, one would not expect to routinely find the infection in the oropharynx if the mode of transmission is primarily genital to oral.

The fact that oropharyngeal infections of *N. gonorrhoeae* were detected in women visiting a dental clinic on a routine visit suggests the possibility of this being a means of identifying asymptomatic infections. The low proportion of positive subjects may partially be attributed to a slightly different subject population in this group when compared to the other two groups. None of the single women admitted to sexual activity, and although the older patients (35 and over) were still capable of childbearing, it is possible that they had reached an age where the mental attitude, in general, is toward not bearing children, particularly if they are multiparous. Because of this possible change in attitude, the exposure of older women may be less frequent and of a different nature than may occur in the younger age group. Another possible cause for the observed difference between the two U. S. military dependent groups may be related to dental care. Many individuals may have brushed their teeth or used some form of cleanser for their mouth and throat just prior to the sample being taken, thereby making the organism more difficult to recover for culture purposes.

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Effect of a Copper–Containing Intrauterine Device on *Neisseria gonorrhoeae in vitro*

Principal Investigators: Michael R. Spence, MAJ, MC
Douglas R. Stutz, MAJ, MSC

Associate Investigator: Wichiyan Panlom

OBJECTIVE: To determine if a copper containing intrauterine device (IUD) inhibits the growth of *Neisseria gonorrhoeae in vitro.*

BACKGROUD: Copper possesses antifertility properties (1). It also inhibits both adult and fetal cell growth in tissue culture (2). Metallic copper and cupric ion either kill or inhibit the growth of *N. gonorrhoeae in vitro* (3).

DESCRIPTION: Thayer–Martin selective media (G.C. Agar Base, hemoglobin VCN and IsoVitaleX, Baltimore Biological Laboratories, Cockeysville, Maryland 21030 U.S.A.) was used for isolation. Determinations of growth inhibition by copper were made on a typing medium developed by Kellogg (4). All plates were incubated at 36°C for 24 or 48 hours in candle jars in an increased CO_2 atmosphere. Electrolytic copper wire, 0.5 mm in diameter, was cut to one inch lengths and sterilized by autoclaving. An electrolytic copper plate was cut into discs approximately 1.5 cm in diameter and sterilized by autoclaving. The copper–containing IUD (Gravigard) was furnished by G.D. Searle (Thailand) Ltd. and was aseptically prepackaged. The strain of *N. gonorrhoeae* used was isolated from the urethral exudate of a male with acute urethritis. The exudate was streaked directly on freshly prepared Thayer–Martin media which was then incubated at 36°C in a candle extinction jar. The culture was inspected at 24 and 48 hour intervals and those colonies with gross morphology resembling *Neisseria* were subjected to Gram staining and tested with oxidase reagent. All colonies of Gram negative diplococci that gave positive tests with oxidase reagent were verified as *N. gonorrhoeae* by sugar fermentation.

The copper wires, discs and copper–containing IUD were placed on Kellogg's typing medium previously streaked with gonococci and incubated at 36°C for 48 hours under an increased CO_2 atmosphere.

PROGRESS: The copper wires and discs inhibited the growth of *N. gonorrhoeae* as previously described (3). In addition, the copper coil portion of the Gravigard IUD also inhibited the growth of the organism (Figure 1). When the IUD was removed from the medium (Figure 2), it was observed that the presence of the plastic portion of the IUD had also prevented growth; however, we believe that this was not due to the plastic, but to the fact that the medium had been covered as no zone of inhibition was observed in these areas. A similar absence of growth has also been observed when plastic tubing was placed on the medium. The area surrounding the copper coil (approximately 2 mm on either side of the IUD), however, did not have bacterial growth.

SUMMARY: This preliminary study suggests that the copper–containing IUD may play a useful role in preventing gonorrhea. The solubilization of copper has been estimated at one microgram of copper per day (3) which is within the known bactericidal range *in vitro* suggesting that growth of *N. gonorrhoeae* may be inhibited for a period of time after the IUD has been inserted in the uterus.

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Figure 1. Inhibition of the growth of gonococci by a copper-containing intrauterine device (Gravigard). A zone of inhibition indicated by arrows at the margins may be seen surrounding the copper-containing portion of the IUD

Figure 2. Inhibition of the growth of gonococci by a copper-containing intrauterine device (Gravigard). The IUD has been removed to better demonstrate the zone of inhibition. Although growth has been inhibited in the area where the plastic was present (arrows), it is believed that this is not due to any peculiar characteristic of the plastic, rather to the media being covered as discussed in the text.

Microbial Flora Present in the Anterior Urethra of Venereal Disease Patients

Principal Investigators: Gary D. Phillips, CPT, MSC
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Somnuk Vibulsek, COL, MC, RTA[1]

Associate Investigators: Tatsanee Occeno, B.S.
Kanachana Leelasiri, B.Sc.
Wichiyan Paniom

OBJECTIVE: It is the purpose of this study to determine the microorganisms present in the anterior urethra of males attending a Venereal Disease Clinic.

BACKGROUND: Microorganisms isolated from patients with urethritis may be clearly pathogenic or they may be organisms which are commonly associated with normal flora of the skin. Gram positive cocci have frequently been implicated in non-specific urethritis (NSU) and urinary tract infections (1, 2, 3). Anaerobic organisms have been reported by various investigators as pathogens of the genito-urinary tract (4,5).

DESCRIPTION: The patient population consisted of 72 men attending the Venereal Disease Clinic of the Royal Thai Army Hospital, Bangkok, Thailand. Patient's ages ranged from 17-24 years (average 22.8). The patients were separated into two groups. Group I consisted of all patients presenting with symptoms of urethritis. Group II were those patients with venereal disease other than urethritis. Both groups were sexually active, and of the same age and socioeconomic position. Specimens for culture were obtained from the anterior urethra of all patients using a calcium alignate naso-pharyngeal swab. The swab was roll-streaked on various plated media immediately after it was obtained. A duplicate specimen was obtained from the anterior urethra for the culture of anaerobic organisms. All clinical specimens were inoculated into three basic media: modified Thayer-Martin (TM) media (6), 5% sheep blood agar plates (BAP), and anaerobic broth media. The anaerobic media consisted of a pre-reduced broth (Basal Medium - PY -Peptone Yeast) which was used as a transport medium until the sample could be transferred to chopped meat media (7). The cultures were transferred to the laboratory at the close of the clinic (normally less than two hours after collection) where they were streaked for isolation.

The plate media were incubated at 35°C under increased CO_2 tension (candle extinction jar). The samples for anaerobic culture were removed from the basal media by piercing the rubber stopper with a sterile needle and aspirating the fluid with a syringe. The specimen was then inoculated into chopped meat broth media under a stream of CO_2 gas rendered free of trace amounts of oxygen by passing through a heated copper oven (Sargent Welch Scientific Co.). The tubes were sealed with rubber stoppers and shrinkable cellulose sealing bands (Pharmaceutical Laboratory, Perry Point, MD.). Simultaneously a 0.5 ml portion of the basal media was inoculated onto duplicate BAP's and streaked for isolation. The plates were incubated in an oxygen free atmosphere using Gas-Paks (Baltimore Biological Laboratories, Cockeysville, Maryland) at 37°C for 48 hours. When colonies developed they were subcultured to biochemicals for identification. The chopped meat broth cultures were observed for a maximum of two weeks and then discarded if bacterial growth was not detected by Gram stain. If growth developed they were subcultured to BAP's and incubated anaerobically at 37°C for 24-48 hours. Colonies isolated on BAP's were further subcultured to biochemicals for identification. All biochemical subcultures were observed for a maximum of 14 days before discarding.

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All aerobic plates were examined after 24 hours of incubation for the growth of colonies with morphology resembling that of *Neisseria sp.* In some cultures it was necessary to incubate the plates an additional 24 hours before growth was adequate for evaluation. Suspect colonies were identified as *N. gonorrhoeae* on the basis of morphology, oxidase reaction, Gram stain, and sugar fermentations. Organisms which were inhibited on TM media were evaluated using BAP's. Subsequent identification employed tubed biochemical media. Significance of differences between groups was determined by Chi–square testing employing Yates correction (8).

PROGRESS: There were no significant differences in the recovery of Gram positive cocci between the two groups. Anaerobic organisms were found only slightly less frequently than the Gram positive cocci. They were isolated 10% more often in patients with urethritis as compared to those with other venereal diseases (Table 1). This difference is not significant.

Neisseria gonorrhoeae was found in 40.5% of the patients in Group I. In Group II patients, 5.7% were found to harbor *N. gonorrhoeae*, thus representing the asymptomatic carrier (Table 1).

Two patients were found to have *Staphylococcus aureus* and three had fecal organisms. None of these occurred alone, but were found concomitantly with other Gram positive cocci and anaerobes. There were no significant differences in the isolation of anaerobes and Gram positive cocci in patients with gonococcal urethritis and non–gonococcal urethritis (Table 2).

Table 1. Microorganisms Found in the Anterior Urethra of Urethritis Patients Compared with Those Having Venereal Disease Other Than Urethritis

Organisms Found	V.D. Other Than Urethritis (35 Patients)	Urethritis (37 Patients)
Gram–positive Cocci	29 (83%)	32 (87%)
Anaerobic Organisms	21 (60%)	26 (70%)
Anaerobes and Gram–positive Cocci	18 (51%)	22 (59%)
Neisseria gonorrhoeae	2 (5.7%)	15 (41%)

Table 2. Microorganisms Found in the Anterior Urethra of Gonococcal (GC) Urethritis and Non–gonococcal Urethritis Patients

Organisms Found	G.C. Urethritis (15 Patients)	Non–G.C. Urethritis (22 Patients)
Gram–positive Cocci	4 (27%)	5 (23%)
Anaerobic Organisms	1 (6.7%)	2 (9.1%)
Anaerobes and Gram–positive Cocci	9 (60%)	12 (55%)
Neisseria gonorrhoeae	1 (6.7%)	0
Fecal Organisms	0	3 (14%)

DISCUSSION: Non-specific urethritis is frequently a complaint of males attending military health clinics. The cause of this condition is currently unknown. Gram positive cocci, diphtheroid bacilli, chlamydia, and the T- strain of mycoplasma have all been alleged to be causative agents (6). Gram positive cocci, have been found by some observers to be the cause of NSU. However, we found these organisms present with nearly identical frequencies in both of our study groups. It may be inferred from this observation that while the Gram positive cocci may be pathogenic they are frequently present as opportunist commensals. The susceptibility of the individual may in some way lead to the disease state, but this is unclear at this time.

Anaerobes were found in 60 % of our nonurethritis patients and 70 % of those with urethritis. In another sample population (unpublished observations) consisting of 17 normal healthy males, we found 15 (88%) harboring anaerobes in the urethra. Anaerobes have been found in the urethra of both symptomatic and asymptomatic males attending a venereal disease clinic. These findings confirm the presence of anaerobes in the male anterior urethra. Swenson, et al, found anaerobic bacteria causative in 80 % of female genital tract infections (9). These organisms have also been found in normal vaginal secretions (10). Therefore, they should be considered as part of the normal microbial flora in both the male and female genital tract. They may serve as sources of genito-urinary infections in susceptible sexually active males.

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An Epidemiological Survey of Males with Urethritis Attending a Military V.D. Clinic in Thailand

Principal Investigators: Richard M. Price, SSG
Michael R. Spence, MAJ, MC

Associate Investigator: Wichiyan Paniom

OBJECTIVE: To review data obtained from an informal questionnaire of male patients with a urethral discharge and to correlate this information with their smear and culture results.

BACKGROUND: A Venereal Disease Workshop (SEATO Medical Research Laboratory, June 1974) revealed an increasing frequency of venereal disease among American military forces in Thailand. Little is known of factors influencing this increase. It is the purpose of this study to determine if the sexual and social habits of patients correlate with their smear and culture results.

DESCRIPTION: Data from 139 males presenting to the U. S. Army Dispensary, Camp Samaesan, Thailand, were studied. An informal questionnaire was verbally administered to each patient by a technician.

For all patients a smear of the urethral discharge was prepared, Gram stained and microscopically examined for the presence of Gram negative intracellular and extracellular diplococci morphologically resembling *Neisseria gonorrhoeae*. A culture was obtained by introducing a calcium alginate nasopharyngeal swab into the anterior urethra. Appropriate media were streaked for the isolation of anaerobes, aerobes and *N. gonorrhoeae*.

PROGRESS: The information obtained by questionnaire is currently being correlated with the laboratory results.

Resistance of Gibbons (*Hylobates lar*) to Gonocccal Infection

Principal Investigators: Chaufah Thongthai, Ph. D.[1]
Douglas R. Stutz, MAJ, MSC

Associate Investigators: Stitaya Sirisinha, D.M.D., M.S., Ph. D.[1]
Apinya Asavanig, M.S.[1]
Markpol Tingpalapong, D.V.M.

OBJECTIVE: To determine if the white-handed gibbon (*Hylobates lar*) would serve as a satisfactory host for experimental infection with *Neisseria gonorrhoeae*.

BACKGROUND: Attempts to produce *N. gonorrhoeae* infection in gibbons were previously unsuccessful at SEATO Medical Research Laboratory (1). This study is a continuation of that investigation.

DESCRIPTION: Adult male gibbons were inoculated intra-urethrally with 5×10^6 gonococcal colony forming units (CFU) from urethral exudate of ten male patients. The animals were observed for 30 days with daily urethral cultures obtained throughout the testing period.

Urethral exudate gonococci (GC) were incubated at both 39°C (gibbon body temperature) and at 36°C. After incubation CFU's were determined for both incubation temperatures. For comparison, cultures of type one GC (F 62) were also incubated at 39°C and 36°C and CFU's observed.

The gonococcocidal activity of gibbon blood leukocytes was measured. This was accomplished by incubating a mixture of type one GC and leukocytes at 39°C in the absence of serum.

RESULTS: All four gibbons inoculated with urethral exudate showed elevated leukocyte counts and developed a clear discharge, but yielded negative results for urethral smears and cultures for *N. gonorrhoeae* throughout the 30 days of observation. Urethral exudate gonococci incubated at 39°C showed decreased numbers of CFU when compared with incubation at 36°C. Stock cultures to type one GC (F 62) produced analogous results at both temperatures. In the measurement of the gonococcocidal activity of gibbon leukocytes, it was found that killing was negligible (mean = 53%) at 60 minutes but became significant (mean = 78%) at 120 minutes of incubation.

PROGRESS: We previously reported that type one GC (F 62) incubated as above with human leukocytes were not significantly killed (mean = 40%) at 120 minutes of incubation. That data in combination with the present results suggests that the relatively more efficient gonococcocidal activity of gibbon leukocytes may play a role in the resistance of gibbons to gonococcal infection.

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Clinical Observation of *Vibrio parahemolyticus* Infection in Thailand

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Assistant Investigators:	Chetana Maiphoom[1] Chanpen Srimunta

OBJECTIVE: To determine the clinical pattern of *V. parahemolyticus* gastroenteritis in Thai patients, and to evaluate the efficacy of common antimicrobial agents in the treatment of the disease.

BACKGROUND: Studies on *V. parahemolyticus* infection in Thailand were initiated by SEATO Medical Research Laboratory (SMRL) in 1970. The preliminary study indicated that *V. parahemolyticus* was a major cause of gastroenteritis in adults in Bangkok (1). At the Bumrasnaradura Infectious Disease Hospital, located in Nonthaburi, this organism was isolated from approximately 25% of the diarrheal patients. Throughout the year the marine environment, (sea water, fish, crabs, oysters) was found heavily contaminated with this halophilic bacillus; therefore, sea foods are implicated as the major source of the *V. parahemolyticus* diarrheal outbreaks in this community. The detailed clinical picture of this disease, its mode of transmission in Thailand, and the efficacy of antimicrobial therapy has not previously been described.

DESCRIPTION: All patients admitted to the Infectious Disease Hospital, Nonthaburi, with symptoms of acute gastroenteritis between September 1973 and August 1974 were included in the study. Rectal swabs for bacterial cultures were obtained daily for three consecutive days. Those patients with positive stool cultures for *V. parahemolyticus* were selected for the study.

A detailed history of the illness, and clinical findings were recorded. Blood cultures, leukocyte counts, urinalysis, and serum electrolyte determinations were made on the first day of admission, and subsequently as indicated.

Patients were randomly divided into one control group and two test groups. The control group received a placebo plus symptomatic and supportive therapy. One test group received co-trimoxazole (two adult tablets two times a day for five days) plus symptomatic and supportive therapy. The remaining group received oral tetracyline (40 mg/kg/day for five days) plus symptomatic and supportive therapy. Rectal swabs in each group were obtained and cultured daily for seven days or until cultures were negative for *V. parahemolyticus* for the three consecutive days.

RESULTS: Two hundred and twenty eight patients admitted to the hospital during the study period were found to harbor *V. parahemolyticus* in their diarrheal stools; of these patients 133 were available for clinical evaluation.

Forty three patients were treated with co-trimoxazole, 42 with tetracycline, and 48 were given placebos as a control. All patients were characterized in terms of age, sex, and duration of illness before therapy. These variables were comparable in all three study groups (Table 1).

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† Bumrasnaradura Infectious Disease Hospital, Nonthaburi, Thailand.

Table 1. Description of the 133 Patients Studied by Age, Sex and Duration of Illness

Characteristic	Placebo	Tetracycline	Co–trimoxazole
Age (Years)			
Less Than 20	8	7	10
20–40	28	22	20
40–60	9	10	10
More Than 60	3	3	3
Sex			
Male	30	30	27
Female	18	12	16
Duration of Illness before Therapy			
Less Than One Day	47	40	43
One–Two Days	1	2	-
Total Patients	48	42	43

Table 2. Clinical Findings of *V. parahemolyticus* Gastroenteritis in 133 Patients

Clinical Features	No. of Patients	%
Symptoms and Signs		
Abdominal Pain	132	99
Abdominal Distension	59	44
Abdominal Tenderness	6	5
Vomiting	118	89
Fever	59	44
Headache	43	32
Characteristics of the Stool		
Watery	102	76
Semisolid	30	22
Bloody	1	1
Mucus	1	1

The disease was characterized by acute, profound diarrhea with nausea and vomiting. The predominant symptom was colicky abdominal pain. Fever and headache were observed to a lesser degree. The stool was watery or semisolid without blood or mucus in the majority of cases (Table 2).

Antimicrobial Sensitivity: Sensitivity profiles are presented in (Table 3). Using the standardized single disc method of Bauer and Kirby (2), it was found that the majority of the isolates (78% to 100%) were sensitive to chloramphenicol, tetracycline, co-trimoxazole, neomycin, erythromycin and streptomycin. Only 6% and 62% of the vibrios tested were sensitive to ampicillin and colistin, respectively.

Response to Antimicrobial Agents: Antimicrobial therapy trials comparing oral tetracycline, co-trimoxazole and placebo were performed. There was no great difference in terms of clinical response (Table 4) or bacteriological response (Table 5) among the three groups studied. The majority of patients in each group had negative vibrio stool cultures after four days of therapy.

DISCUSSION: In this study only the severely ill patients requiring hospitalization are presented. The clinical syndrome which we observed in these patients may represent only the severe form of the infection. A complete clinical picture of the mild form of the disease needs to be described. Dehydration was not as severe as that seen in infection with *Vibrio cholera*. Intravenous fluid therapy was required only for the first few days of the illness. Localization of the infection in the lumen of the intestine is suspected due to the presence of diarrhea without bacteremia, leukocytosis or toxic symptoms (Table 6). Previous experiments on the pathogenicity of *V. parahemolyticus* using the infant rabbit model indicated that the organism elaborated toxic substances, presumably enterotoxins, into the intestinal fluid (1). Enterotoxins may play a major role in the pathogenesis of this disease.

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Table 3. Sensitivities of 228 Strains of *V. parahemolyticus* to Eight Antimicrobial Agents

Antimicrobial Agents	Sensitive		Intermediate Sens.		Resistant	
	No. Strain	%	No. Strain	%	No. Strain	%
Co-trimoxazole	228	100	—	—	—	—
Tetracycline	197	86.4	31	13.6	—	—
Chloramphenicol	228	100	—	—	—	—
Ampicillin	2	0.9	12	5.3	214	93.8
Neomycin	98	43	128	56.1	2	0.9
Colistin	70	30.7	72	31.6	86	37.7
Streptomycin	36	15.8	141	61.8	51	22.4
Erythromycin	167	73.3	60	26.3	1	0.4

Table 4. Clinical Response to Antimicrobial Therapy in 133 Patients

Regimens	No. of Patients	No. improved, Days after Treatment							
		1	2	3	4	5	6	7	Unknown*
Placebo	48	—	5	13	11	10	4	—	5
Tetracycline	42	1	9	10	14	2	—	2	4
Co-trimoxazole	43	1	5	15	12	7	1	—	2

*Unknown = Patients excluded from the study because of incomplete study schedule.

Table 5. Bacteriological Response to Antimicrobial Therapy in 133 Patients

Regimens	No. of Patients	No. with Positive Stool Culture, Days after Treatment						
		2	3	4	5	6	7	Unknown*
Placebo	48	7	20	14	6	—	—	1
Tetracycline	42	10	18	12	—	1	—	1
Co-trimoxazole	43	12	19	7	3	1	1	—

*Unknown = Patients excluded from the study because of incomplete study schedule.

Table 6. Laboratory Findings of *V. parahemolyticus* Gastroenteritis

Laboratory Findings	No. of Patients	%
Total Leukocyte Count (per cu mm.)		
Less Than 8,000	63	47.7
8,000–10,000	32	24.2
10,000–15,000	30	22.7
More Than 15,000	7	5.3
Total Number of Patients Tested	132	
Serum Sodium (MEq/L)		
Less Than 130	0	
130–150	83	79.8
More Than 150	21	20.2
Total Number of Patients Tested	104	
Serum Potassium (MEq/L)		
Less Than 3.5	12	11.5
3.5–5.5	88	84.6
More Than 5.5	4	3.8
Total Number of Patients Tested	104	
Blood Culture (No growth)	133	100.0

Cholera Study at Samutsongkram

Principal Investigators: Vichai Sangkasuwan, LTC, MC, RTA
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OBJECTIVE: To investigate the carrier state of cholera in apparently healthy persons.

BACKGROUND: According to the data presented in 1974 by the Ministry of Public Health, cholera has occurred throughout the year in 33 of the 71 provinces. This gives the impression that cholera might have become endemic in some areas of Thailand. Most patients with cholera are free of *V. cholerae* within two weeks and there appears to be no true carrier state. It is suspected, however, that direct man to man infection might play an important role in spreading the disease.

Samutsongkram was selected as a study site because the first case of the cholera "season" (late cool dry to early hot dry seasons) has usually been reported from that area.

DESCRIPTION: An attempt has been made to isolate *V. cholerae* from apparently healthy persons in Samutsongkram and also from patients recovering from cholera. Water samples were collected from the nearby river and were subject to similar isolation attempts.

An attempt has also been made to isolate *V. cholerae* from the stools of patients convalescing from diseases other than diarrhea, after receiving a cholagogue (Veracolate).

PROGRESS: The number of stool and water specimens collected between June 1974 and March 1975 and the results of the isolation attempts are shown in Table 1.

DISCUSSION: It was rather difficult to follow the patients convalescing from cholera at home in order to get the stool specimens for re-examination. Lack of cooperation of the patient was also a problem. It is too early yet to draw any conclusion from the data collected so far.

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Table 1. Cholera Study (Samutsongkram)

Month Year	Fecal Specimens						Water Specimens		
	Healthy Persons			Convalescent Patients					
	No.	*V. cholerae*	NAG[a]	No.	*V. cholerae*	NAG	No.	*V. cholerae*	NAG
June 1974	102	0	3 (3)[b]				5	0	5
July	103	0	2 (2)	44	0	0	6	0	6
August	143	0	7 (5)	87	0	5 (6)	8	0	8
September	144	0	9 (7)	47	0	9 (20)	6	0	6
October	134	0	20 (15)	65	0	7 (10)	0	0	0
November	113	0	3 (3)	67	0	3 (5)	0	0	0
December	102	0	13 (13)	61	0	3 (5)	0	0	0
January 1975	121	0	43 (35)	66	0	20 (3)	4	0	4
February	97	0	31 (30)	56	0	10 (20)	7	0	7
March	98	3[c]	27 (27)	67	0	3 (5)	8	0	8

a. Non-agglutinable vibrios.
b. Number positive (percent).
c. *V. cholerae*, biotype Eltor, Serotype Ogawa (Contact cases suspected).

Detection of Specific Bacterial Antigen by Counterimmunoelectrophoresis (CEP)

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Douglas R. Stutz, MAJ, MSC

Associate Investigator: Mr. Thamma Sakulkaipeera

OBJECTIVE: To detect specific bacterial antigen in infected body fluids by CEP and to compare the presence of antigen with bacteriological studies of the same specimens.

BACKGROUND: Pneumococcal and *Hemophilus influenza* b capsular antigens have been detected in the cerebrospinal fluid (CSF) of patients with purulent meningitis at Children's Hospital. *H. influenza* b antigen has also been detected in subdural effusions from patients with *H. influenza* b meningitis and in the pericardial fluid from a patient with a pericardial effusion, while pneumococcal antigen has been detected in pleural fluid obtained from patients with pneumococcal empyema.

Bacterial antigen has been detected when Gram's stain and culture results have been negative, thus providing an etiologic diagnosis despite negative bacteriological studies.

DESCRIPTION: CEP was carried out on a variety of body fluids, CSF, subdural fluid, pleural fluid and serum. CEP using commercial antisera against *H. influenza* b, pneumococcus and meningococcus type A – D; and antisera prepared in rabbits against *Staphylococcus aureus* was carried out by a previously described method (1).

PROGRESS: *H. influenza* b antigen was detected in the initial CSF of 16 patients with purulent meningitis (Table 1). Eight patients had positive Gram's stain and cultures for *H. influenza* b. Four patients had negative Gram's stains.

Table 1. Detection of *H. influenza* b Antigen

Fluid Source	Positive specimens			Antigen duration (mean days)
	Gram's stain	Culture	CEP	
CSF	8	12	16	3.8
Subdural	1	1	6	9.1
Pleural	0*	0*	4	Not done
Serum	Not done	Not done	4	4.6

*1 Specimen: *Escherichia coli*

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stain and positive cultures, and four patients had both negative Gram's stain and cultures. In eleven patients repeat CSF samples were studied and the mean duration of *H. influenza* b antigen was 3.8 days (range 0 – 11 days). Subdural effusions were present in six patients with *H. influenza* b meningitis. One was Gram's stain and culture positive for *H. influenza* b, the remainder were negative. The mean duration of antigen in subdural fluid, from the day of admission, was 9.1 days (range 1 – 16 days).

H. influenza b antigen was detected in pleural fluid from four patients. One patient had *Escherichia coli* on Gram's stain and culture of the pleural fluid while the remaining patients had negative Gram's stains and cultures. One of these remaining patients had purulent meningitis and a subdural effusion also; *H. influenza* b antigen was detected in these fluids as well.

H. influenza b antigen was found in the sera of four meningitis patients with a mean duration of 4.6 days (range 2 – 6 days) from the day of admission.

Table 2. Detection of Pneumococcal Antigen

Fluid source	Positive specimens			Antigen duration (mean days)
	Gram's stain	Culture	CEP	
CSF	3	5	11	4.3
Subdural	2	2	2	3.5
Pleural	6	7	15	15.2
Serum	Not done	Not done	3	10.7
Pericardial	1	1	1	1

Pneumococcal antigen was detected in the initial CSF fluid of 11 patients with purulent meningitis (Table 2). Three patients had positive Gram's stain and five patients had positive cultures for pneumococci. In nine patients repeat CSF samples were studied and the mean duration of pneumococcal antigen was 4.3 days (range 0 – 11 days). Subdural effusions were present in two patients with pneumococcal meningitis; both were culture positive and Gram's stain positive. Antigen was detected three and four days after admission.

Pneumococcal antigen was detected in the pleural fluid from 15 patients. Six had positive Gram's stain and seven had positive cultures for pneumococci. The mean duration of antigen in pleural fluid was 15.2 days (range 0 – 44 days).

Pneumococcal antigen was present in the serum of two patients with meningitis and one patient with empyema for an average of 10.7 days (range 4 – 15 days) after admission.

One patient with a pyopercardium had pneumococcal antigen detected in the pericardial fluid, which was also Gram's stain and culture positive for pneumococci.

Staphylococcal antigen was detected in the pleural fluid from 13 patients. Only one had a positive Gram's stain and nine had positive cultures. Staphylococcal antigen was detected in fluid from other sites in individual patients as well (Table 3).

Table 3. Detection of Staphylococcal Antigen

Fluid source	Positive specimens		
	Gram's stain	Culture	CEP
Pleural	1	9	13
CSF	0	0	1
Subdural	Not done	0	1
Abscess	1	1	1
Pericardial	1	1	1

DISCUSSION: Employing specific antisera to detect *H. influenza* b, pneumococcal and staphylococcal antigen by counterimmunoelectrophoresis has been useful in providing a rapid etiologic bacterial diagnosis of cases of purulent meningitis and empyema. This technique detects bacterial antigen when Gram's stains and cultures are negative, thus it appears to be a useful adjunct to routine bacteriologic methods. One cross reaction of H. *influenza* b antisera with *Escherichia coli* from pleural fluid was noted, and this may limit the usefulness of the procedure should more frequent cross reactions be observed.

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Antibiotic–Resistant Typhoid Fever

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Associate Investigators: Douglas R. Stutz, MAJ, MSC
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OBJECTIVE: To determine the prevalence and degree of antibiotic resistance among *Salmonella typhi* isolates from typhoid fever patients at Children's Hospital and to correlate the *in vitro* pattern of resistance with the phage types of *S. typhi* and the clinical response to therapy.

BACKGROUND: Antibiotic–resistant strains of *S. typhi* have been isolated from patients with typhoid fever at Children's Hospital. These *S. typhi* strains have resistance patterns and transferable R factor similar to strains isolated from Mexico, India and Vietnam. Chloramphenicol has been ineffective therapy in patients infected with Chloramphenicol–resistant *S. typhi*.

DESCRIPTION: Isolates of *S. typhi* from patients with clinical typhoid fever at Children's Hospital were obtained and disc sensitivities performed by the Kirby–Bauer method, and minimal inhibitory concentrations (MIC) by the plate dilution technique. *S. typhi* isolates from other parts of Thailand, and from Cambodia and South Vietnam were similarly studied.

The presence of R factor in antibiotic resistant strains was determined by the transfer of antibiotic resistance to a sensitive strain of *Escherichia coli*.

Phage typing of the *S. typhi* strains was performed through Dr. E.S. Anderson at the Enteric Reference Laboratory, London, England.

PROGRESS: Forty–four *S. typhi* isolates from Children's Hospital obtained between 28 March 1974 to 11 March 1975 were studied; seventeen (39%) were resistant to Chloramphenicol.

The Kirby–Bauer Disc method showed the resistance Pattern I, Chloramphenicol, Streptomycin, Sulfadiazine, and Tetracycline (C S Su T) in eight strains and the resistance Pattern II (Ampicillin C S Su T) in nine strains.

The disc sensitivities are presented in Table 1 and the minimal inhibitory concentrations to Chloramphenicol, Ampicillin and Trimethoprim/Sulfamethoxazole (TMP/SMZ) are illustrated in Figure 1. In Figure 1 the MIC for TMP/SMZ refers to the μgm/ml of TMP in a 1:20 ratio of TMP:SMZ.

Each Chloramphenicol–resistant strain of *S. typhi* possessed R factor capable of transferring Chloramphenicol resistance to a sensitive E. coli. The strains that were also resistant to Ampicillin transferred both Chloramphenicol and Ampicillin resistance to sensitive *E. coli*.

Vi phage type results are available on *S. typhi* strains obtained from Children's Hospital since November 1973. Their distribution by Chloramphenicol sensitivity is illustrated in Table 2. Most of the *S. typhi* strains resistant to Chloramphenicol but sensitive to Ampicillin have been phage type 53. All of the Ampicillin and Chloramphenicol resistant strains of *S. typhi* have been phage type D 1 (variant).

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Table 1. Antibiotic Disc Sensitivity of *Salmonella typhi* strains (44)

Antibiotic	Sensitivity (percent)
TMP/SMZ	100%
Kanamycin	100%
Gentamicin	100%
Cephalothin	100%
Ampicillin	80%
Chloramphenicol	61%
Tetracycline	61%
Sulfadiazine	30%
Streptomycin	0%

The 10 patients with phage type D 1 (variant) came from scattered locales in Bangkok and nine were hospitalized during the months of May, June and July 1974. Initial therapy with Chloramphenicol or Ampicillin was ineffective in these patients; however, therapy with Trimethoprim/Sulfamethoxazole resulted in satisfactory clinical improvement.

Twenty five strains of *S. typhi* from Vietnam and ten strains of *S. typhi* from Siriraj Hospital, Bangkok, Thailand were confirmed to be resistant to Chloramphenicol by disc sensitivity and MIC. All of the strains exhibited resistance Pattern I, C S Su T. No Ampicillin resistance was detected.

One *S. typhi* strain from Cambodia, and two from Songkla, Thailand were sensitive to Chloramphenicol. One of seven strains of *S. typhi* from the Bumrasnaradura Infectious Disease Hospital exhibited resistance Pattern I, while the remainder were sensitive to Chloramphenicol. All of the 21 *S. typhi* strains from Chiangmai were sensitive to Chloramphenicol and Ampicillin.

DISCUSSION: The emergence of Chloramphenicol–resistant *S. typhi* strains with demonstrable *in vitro* and *in vivo* resistance which was first noted in 1973 has continued and a number of strains have been resistant to Ampicillin as well. All strains have been sensitive to TMP/SMZ and satisfactory clinical responses have been observed with this drug. The potential emergence of resistance of *S. typhi* to TMP/TMZ, remains; however, since resistance to TMP/SMZ is seen in other enteropathogens. Approximately 10% of Shigella isolates at SEATO Laboratory during the past year have demonstrated *in vitro* resistance (disc method) to TMP/SMZ. Continued surveillance of antibiotic resistance patterns of *S. typhi* will continue.

The association of certain phage types with antibiotic resistant strains of *S. typhi* suggests that certain phage types are more likely to be associated with R factor. The phage type associated with antibiotic–resistant strains of *S. typhi* are different than phage types noted in Mexico and in Vietnam and determination of the phage type may prove useful in determining the source of infection in the future.

Table 2. Distribution of Vi Phage Phage Types by Sensitivity (53 *S. typhi* strains)

Phage type	Chloramphenicol sensitive	Chloramphenicol resistant
A	5	
D2	5	
M1	4	1*
53	4	13*
E10	3	
Degraded Vi (7)	2	
E1	1	
E2	1	
E3	1	
D6	1	
Degraded Vi (8)	1	
Vi negative		1*
D 1 variant		10**
Total	28	25

* Pattern I: C S Su T
** Pattern II: A C S Su T

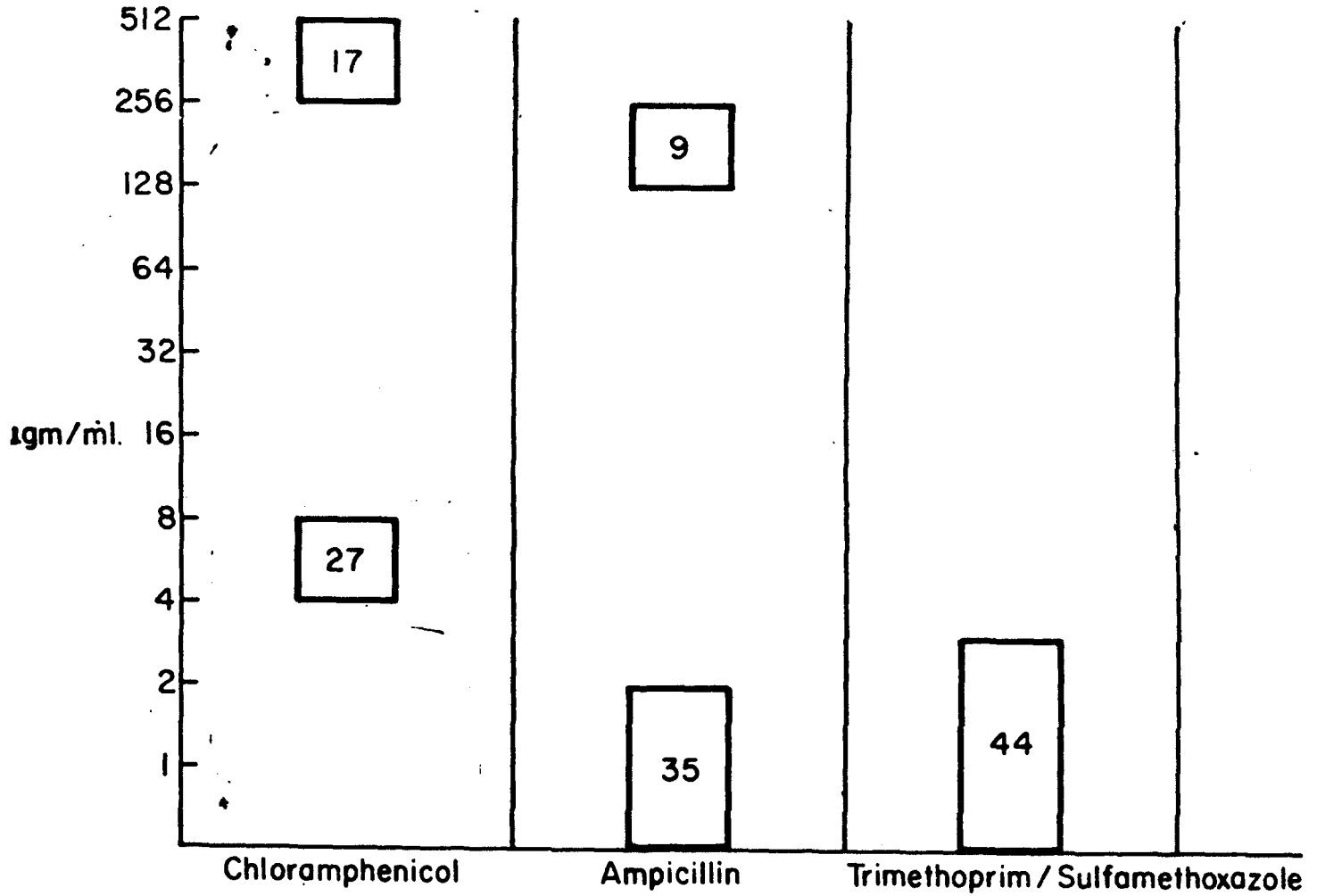

Figure 1. Range of Minimal Inhibitory Concentrations (for 44 S. typhi Strains)

Frequency of Glucose–6–Phosphate Dehydrogenase Deficiency (G–6–PD) among Infectious Disease and Control Patients at Children's Hospital

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OBJECTIVE : To determine the frequency of erythrocyte G–6–PD deficiency among patients hospitalized with various infectious diseases and outpatients free of serious disease, seen at Children's Hospital.

BACKGROUND : A high frequency of G–6–PD deficiency has been noted among Thai children with typhoid fever at Children's Hospital. Additional children hospitalized with infectious diseases including bacterial empyema, meningitis and osteomyelitis, tuberculous meningitis and typhoid fever were studied in addition to outpatient controls to determine the frequency of G–6–PD deficiency.

PROGRESS : Children with bacteriologically confirmed diagnoses of empyema, meningitis, osteomyelitis and typhoid fever had G–6–PD determinations performed by the methemoglobin elution technique of Gall (1).

Children with the clinical diagnosis of tuberculous meningitis and supportive skin test and cerebrospinal fluid chemistries were also studied as were 100 outpatient children judged by history and physical examination to be free of serious bacterial disease.

Table 1 presents the frequency of G–6–PD deficiency according to bacterial etiology and sex of the patients.

DISCUSSION : A high frequency of G–6–PD deficiency was noted in patients with typhoid fever (45 %), pneumococcal empyema and meningitis (36 %), and tuberculous meningitis (26 %). A smaller number of patients with Staphylococcal or *Hemophilus influenzae* b infections had no apparent increased prevalence of G–6– PD deficiency (0–10 %). The overall frequency of G–6–PD deficiency among outpatient controls was 11 %.

The association of G–6–PD deficiency with typhoid and pneumococcal infections is analogous to the association of sickle cell anemia with Salmonella osteomyelitis and pneumococcal infections. An impaired reticuloendothelial system as a result of hemolysis has been suggested as a factor in the susceptibility of sickle cell patients to these infections. Defective bacteriocidal activity of the leukocytes from patients with erythrocyte G–6–PD deficiency has been noted when the G–6–PD activity of these leukocytes is absent or markedly depressed (less than 25 %). An overburdened reticuloendothelial system as a result of hemolysis or defective bacteriocidal activity of leukocytes from G–6–PD deficiency patients are possible explanations for this association of erythrocyte G–6–PD deficiency and some bacterial infections.

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Table 1. Frequency of G–6–PD Deficiency by Bacterial Etiology and Sex

Study subjects	Sex	No. tested	G–6–PD deficiency		Frequency (%)
			Homozygous	Heterozygous	
Typhoid fever	M	19	7	0	37
	F	25	4	9	52
Pneumococcal infections	M	17	6	0	35
	F	8	0	3	37
Tuberculous infections	M	14	3	0	21
	F	9	1	2	33
Staphlococcal infections	M	4	0	0	0
	F	5	1	0	20
H. influenza b infections	M	6	0	0	0
	F	2	0	0	0
Outpatient control	M	55	8	0	15
	F	45	0	3	7

Surveillance Study of Plague in Thailand

Principal Investigators: Vichai Sangkasuwan, LTC, MC, RTA
Manit Kanvallee, Fly. Off., VC, RTAF
Krasare Nabnean, LT, MSC, RTA
Varakorn Suriyamongkol, Ensign, MSC, RTN

OBJECTIVE: To investigate the existence of plague in rodents and dogs. This represents a surveillance study.

BACKGROUND: It is believed that besides the Republic of Vietnam, the infection exists in some parts of Burma and Cambodia as well. While there still are cases of plague in neighboring countries, none has been reported in Thailand during the last 30 years.

DESCRIPTION: Blood samples were collected from trapped rodents and dogs from some areas of Aranyapradet. A hemagglutination (HA) test was performed on these sera at the SMRL (Table 1).

PROGRESS: Only one trip has been made to eastern Thailand to date. A small number of rodents were trapped and a number of dogs were bled. A still smaller number of fleas were collected from the 16 trapped rodents in all. All fleas are of the species *Xenopsylla cheopis* and all were collected from *R. exulans*. Some more trips will be made in the future.

Table 1. Result of HA Test for Plague, Aranyapradet (March 1975)

Animal Sera	No. of Specimens	No. Positive
Dog	40	0
Rodent	21	0

Mycoplasma pneumoniae Study

Principal Investigators: Vichai Sangkasuwan, LTC, MC, RTA
Krasare Nabnean, LT, MSC, RTA

OBJECTIVE: To study the occurrence of *Mycoplasma pneumoniae* infection in patients suffering from lower respiratory tract infection.

BACKGROUND. Approximately 3 – 5 % of apparently healthy Thai subjects studied harbor *Mycoplasma pneumoniae* in their throat (unpublished data, Thai Component, SMRL). The organism might invade the lower respiratory tract leading to bronchitis or pneumonia or these people can serve as carriers.

DESCRIPTION: Two samples of blood collected at a 10–14 day interval from patients with bronchitis or pneumonia are to be tested for evidence of the infection by a complement fixation (CF) test.

RESULT: To date only four pairs of sera from cases of acute bronchitis and three pairs of sera from cases of pneumonitis were obtained from the outpatients of Pramongkutklao Hospital. The CF test is underway.

PARASITIC DISEASES OF MAN AND ANIMALS

Ecology of Bancroftian Filariasis

Principal Investigators: Charles L. Bailey, CPT, MSC
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Assistant Investigators: Kol Mongkolpanya
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OBJECTIVE: To investigate the ecology of bancroftian filariasis in rural areas of Sangkhlaburi district, Kanchanaburi Province. Specific objectives include the following:

1. To identify the vector(s) of *Wuchereria bancrofti* by A) the demonstration of filariae in wild-caught mosquitoes and B) by feeding laboratory-reared strains of potential vector species on known microfilaria-carriers.

2. To determine the prevalence of human infections and the periodicity of microfilaremia, applying the techniques of direct chamber counting and membrane filtration for the isolation of microfilariae.

3. To collect information on the distribution, larval habitats and bionomics of suspected vector species and to obtain correlated series of larvae, pupae and adults of these mosquitoes for taxonomic studies.

4. To evaluate the clinical expression of human infections.

DESCRIPTION: In 1970 Harinasuta and associates (1) described an endemic focus of bancroftian filariasis in rural villages located near the headwaters of the Kwai River in the Sangkhlaburi district of Kanchanaburi Province. Prevalence rates of infection with *W. bancrofti* up to 30 per cent were observed in some villages, and many cases of filarial hydrocoele were reported. Bancroftian filariasis is rarer in Thailand than the type caused by *Brugia malayi;* moreover, in this area it differed from the type usually seen in Southeast Asia in that microfilaremia was nocturnally subperiodic, with peaks near 2000 hours, but with microfilariae present in significant numbers in the peripheral blood during daylight hours. Infective stage larvae of *W. bancrofti* were found in wild-caught mosquitoes belonging to the *Aedes (Finlaya) niveus* complex. The females of *A. inveus* and possibly 20 other closely related species are so similar that they cannot be differentiated with certainty at the present time; these mosquitoes are among the most common diurnal man-biting mosquitoes in the forested areas of Southeast Asia, Harinasuta *et al* also reported finding *Aedes (Finlaya) harveyi, Anopheles maculatus, Anopheles minimus* and *Anopheles vagus* infected with immature filarial larvae. Subperiodic *W. bancrofti* infections transmitted by *Anopheles minimus flavirostris* and species of the *Aedes niveus* complex have also been reported from the Philippines by Cabrera and Rozeboom (2) and *Anopheles leucosphyrus* was identified as the vector of *W. bancrofti* in Sarawak (3).

The detection of microfilaremia, most often by examination of thick films prepared from 20 to 40 c. mm. of blood obtained from the finger, has been commonly relied upon to determine filariasis prevalence rates. The thick film technique has the advantage of being easy to use in the field; however, in recent years it has been shown that prevalence rates and the apparent age distribution of microfilaremia, based upon this survey method, have been imprecise (4). A relatively new technique, that has been shown to be as sensitive as the thick film and as easy to perform under field conditions, is that of direct counts of microfilariae in specially constructed chambered slides (5). Another new survey procedure, the isolation of microfilariae by filtration of blood through Millipore (6) or Nucleopore filters (7), has yielded higher positivity rates, in areas of nocturnally periodic infections, from daytime bloods than the standard thick blood film taken at night during microfilaremia peaks (8). The value of this technique in identifying

microfilaremia carriers with low density infections is obvious. These newer survey techniques should prove valuable in clinical practice, in the evaluation of control and treatment schemes and in attaining a better understanding of the mechanisms of filarial transmission. During the previous reporting period seven villages located in semiforested areas of Sangkhlaburi district were surveyed for microfilaremia. Five of these villages—Kupadu, Lawa, Nithae, Nong Padong and Wang Kalang—were selected as sites for further studies because their high microfilaremia rates and/or accessibility.

PROGRESS: Between July and December 1974, 6169 mosquitoes were caught in 1832 man—hours of collections from human hosts, made during both daylight and evening hours, in the five study sites. Eight genera of mosquitoes, comprising 86 species, were represented in these collections; however *Aedes albopictus, Armigeres annulitarsis* and members of the *Aedes niveus* complex together formed the major portion of those collected. A total of 5141 mosquitoes were dissected, and 45 mosquitoes, belonging to six species (*Aedes niveus* species "A", *Aedes desmotes, A. mediopunctatus, A. gardneri A. imprimens* and *Armigeres annulitarsis*), were found naturally infected with filarial larvae (Table 1). Identifications of the mature larvae are not yet complete, but some are characteristic of *W. bancrofti* while others appear to be species of *Setaria, Diptelonema and/or Breinlia.*

Surveys for larval mosquitoes were made in the five study sites between July 1974 and March 1975. Larvae of 82 species, belonging to 14 genera of mosquitoes, were collected from a variety of habitats within the vicinity of the five villages. Dense thickets of bamboo are present around Kupadu, Lawa and Nong padong, and mosquitoes which breed in bamboo nodes, such as *Aedes (Stegomyia), Aedes (Finlaya)* and *Armigeres (Leicesteria)*, were especially numerous there. This accounts for the abundance of adults of *A. albopictus, Armigeres annulitarsis* and members of the *Aedes niveus* complex caught in biting collections made during this period in those villages. During the rainy season (July—November), larvae of *Anopheles balabacensis,* the principal malaria vector in Thailand, were present in ground pools in all five of the villages. The domestic mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus,* were not present in the more isolated villages of Kupadu, Lawa and Nong Padong, which, incidentally, had the highest microfilaremia rates of the five study sites. On the other hand, larvae of *A. aegypti* and *C. quinquefasciatus* were found breeding on the premises of 72% and 24%, respectively, of the houses in Nithae, which is the most urbanized and had the lowest microfilaremia rate of the five villages.

Colonized strains of *Aedes aegypti, A. albopictus, A. togoi, Armigeres annulitarsis,* and *Culex quinquefasciatus* were fed upon known microfilaria carriers from the Sangkhlaburi district during this period. Of these five species, only *Aedes togoi* and *Culex quinquefasciatus* developed infections with *W. bancrofti* (Table 2).

Between August 1974 and March 1975, a comparison of the relative sensitivity of the 20 c. mm. thick film, 20 c. mm. counting chamber and the 1.0 ml. membrane filtration techniques for detecting microfilaremia was made in the villages of Kupadu and Nong Padong. Blood specimens were obtained from 117 individuals for comparison of the three techniques; the results are shown in Table 3. Microfilaremia rates by age and sex, obtained by the membrane filtration technique, are shown in Tables 4 and 5. A further evaluation of the three techniques was made in a study of the periodicity of microfilaremia in 10 villagers found positive during the earlier survey. Blood was taken at two hour intervals over a 24 hour period, and the microfilaremia densities at each interval, as measured by each of the three techniques, are given in Table 6—8. The mean values of microfilaremia periodicity determined by membrane filtration for the 10 patients are shown in Figure 1. The nocturnally subperiodic character of the Sangkhlaburi strain of *W. bancrofti* is obvious, for at no time was the microfilaremia level less than 20 per cent of peak count. It appears from the above data, that the direct chamber count is at least as sensitive as the standard thick film, with the advantage of providing an immediate result without the necessity of drying and staining slides. The disadvantage is the need for microscopy at the site of specimen collection. Membrane filtration, although venipuncture is required, is still convenient enough for routine field use, and is more sensitive than either the thick film or the counting chamber. The value of this technique in low density infections is illustrated by the case of periodicity patient No. 10 (Table 8).

Seventy—five individuals in the village of Kupadu submitted to full physical examinations during the course of those surveys. Only five of these yielded positive findings as summarized in Table 6.

Table 1. Mosquitoes Found Infected with Filarial Larvae—Sangkhlaburi, 1974

Species	Mupa Du		Nong Pa Dong		Lawa		Nithae		Wong Ka Lang		Totals	
	No. Coll.	No. Pos.	No. Coll.	No. Pos.	No. Coll.	No. Pos.	No. Coll.	No. Pos.	No. Coll.	No. Pos.	No. Coll.	No. Pos.
Aedes (S) gardnerii	19	1	29	1	45	11	10	1	19	3	122	17
Aedes (S) desmotes	55	2	37	0	42	3	8	0	3	0	145	5
Aedes (S) mediapunctatus	45	1	25	3	23	0	3	1	2	0	98	5
Aedes (F) niveus "A"	421	2	83	1	134	3	6	0	21	0	665	6
Aedes (E) imprimens	63	0	51	2	12	0	0	0	1	0	127	2
Armigers (L) annulitarsis	333	1	125	3	122	1	111	1	5	0	696	6
Armigeres (L) flavus	7	0	0	0	9	1	6	0	0	0	22	1
Mansonia (M) dives	11	0	15	0	1	0	4	0	32	3	63	3
Totals	954	7	365	10	388	19	148	3	83	6	1938	45

Table 2. Mosquitoes Fed on *Wuchereria bancrofti* Cases—Sangkhlaburi, 1974

Species	No. Fed	No. Dissected	No. Infected	Percent Infected	No. Larvae
Aedes aegypti	271	207	0	0.0	0
Aedes albopictus	62	46	0	0.0	0
Aedes togoi	138	45	23	51.1	80
Armigeres annulitarsis	27	19	0	0.0	0
Culex quinquefasciatus	333	184	9	4.9	12

Table 3. Results of Comparison of Three Techniques for Detecting Microfilaremia—Kupadu and Nong Padong, 1974

Technique	No. Patients	No. Positive	Percent Positive
Thick Film (20 c.mm. blood)	117	20	17
Counting Chamber (20 c.mm. blood)	117	22	19
Membrane filtration (1 ml. blood)	117	31	26

Table 4. Results of Examinations for Microfilaremia by Age, Using Membrane Filtration Technique—Kupadu and Nong Padong, 1974

Age Group (Years)	Number Examined	Number Positive	Percent Positive
0–3	5	2	40
4–6	8	0	—
7–10	21	2	10
11–15	21	4	19
16–20	13	3	23
21–30	22	7	32
31–40	7	2	29
41–50	12	5	42
50+	8	7	88
Total	117	32	27

Table 5. Results of Examinations for Microfilaremia by Sex, Using Membrane Filtration Technique—Kupadu and Nong Padong, 1974

Sex	Number Examined	Number Positive	Percent Positive
Male	73	17	23
Female	44	15	34
Total	117	32	27

Table 6. Twenty–four Hour *W. bancrofti* Microfilariae Counts in 10 Carriers, by Thick Film Technique

Case No.	Microfilariae counts at hours/20 C.mm. blood											
	1200	1400	1600	1800	2000	2200	2400	0200	0400	0600	0800	1000
1	3	3	7	2	7	3	5	11	10	6	1	3
2	8	20	11	11	19	14	11	11	3	6	3	8
3	5	10	15	20	8	24	13	8	11	5	4	9
4	13	12	25	53	59	82	48	65	57	47	1	1
5	1	4	8	7	3	5	11	8	5	6	1	1
6	3	3	2	1	3	0	1	2	0	1	0	2
7	16	34	46	21	26	51	67	31	16	9	6	29
8	23	29	82	40	67	71	31	56	10	14	6	8
9	5	8	18	23	25	16	9	15	21	13	3	7
10	0	0	0	1	0	0	0	0	0	1	0	0

Table 7. Twenty–four Hour *W. bancrofti* Microfilariae Counts in 10 Carriers, Determined by Counting Chamber Technique

Case No.	Microfilariae counts at hours/20 c.mm. blood											
	1200	1400	1600	1800	2000	2200	2400	0200	0400	0600	0800	1000
1	2	1	1	3	4	12	6	4	7	4	3	2
2	10	19	9	20	23	6	9	10	10	3	5	13
3	4	25	23	23	16	23	16	10	8	1	7	3
4	8	15	31	40	48	62	42	48	49	46	15	11
5	3	2	2	5	5	4	2	7	2	2	4	1
6	6	3	4	3	3	1	1	1	0	1	0	1
7	17	27	53	27	29	49	40	12	23	10	14	17
8	23	30	64	41	56	52	38	45	19	23	7	3
9	6	7	14	14	17	8	15	17	15	11	4	8
10	2	1	0	2	5	2	0	1	1	1	0	1

Table 8. Twenty–four Hour *W. bancrofti* Microfilariae Counts in 10 Carriers, Determined by Membrane Filtration Technique

Case No.	Microfilariae counts at hours/1 ml. blood											
	1200	1400	1600	1800	2000	2200	2400	0200	0400	0600	0800	1000
1	109	96	194	245	334	271	433	318	270	200	128	64
2	444	924	560	1049	966	549	521	456	290	231	198	496
3	371	990	1171	1231	601	727	621	399	601	231	198	257
4	409	685	1560	2282	1789	3124	2653	2462	2841	2244	928	871
5	126	44	327	394	316	294	279	283	183	295	99	109
6	132	210	157	120	197	75	107	85	98	57	49	142
7	861	1824	2508	1686	1231	2102	2093	1917	616	769	756	1310
8	925	1672	3239	2358	3681	2891	2306	2950	877	945	273	334
9	439	564	749	1101	1070	1212	675	1036	473	1033	620	294
10	8	10	22	16	23	43	15	22	2	14	13	5

Table 9. Summary of Clinical Findings in Kupadu Villagers Suggestive of Filariasis

Case No.	Age	Sex	Finding	Microfilariae Present
1	52	M	Large hydrocoele	Yes
53	25	M	Large inguinal nodes, no other apparent cause	No
55	25	M	Large inguinal nodes, no other apparent cause	No
58	21	M	Thickened spermatic cord	No
62	52	M	Thickened spermatic cord	Yes

The pathology normally associated with infections of *W. bancrofti* is apparently at a very low level in this particular endemic area and seems to be limited to minor genital abnormalities in males. No cases of elephantiasis have been observed in the study villages.

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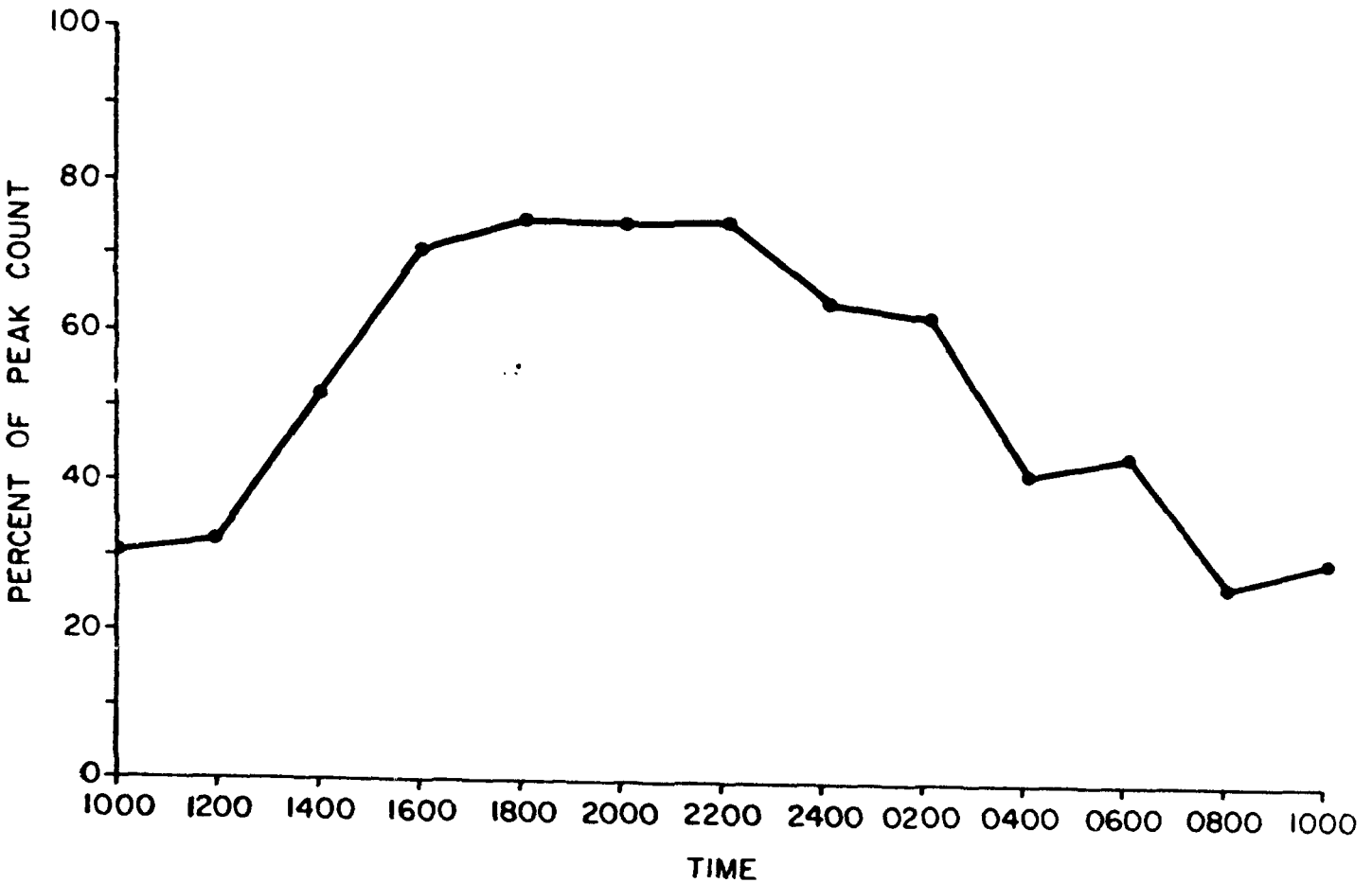

FIGURE 1. MEAN VALUES OF MICROFILARIA DENSITY FOR 10 PATIENTS, DETERMINED AT HOURLY INTERVALS BY 1 ML. MEMBRANE FILTRATION

Evaluation of Experimental Antimalarial Drugs in Rhesus Monkeys Infected with *Plasmodium cynomolgi*

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OBJECTIVE: To evaluate the effectiveness of selected experimental drugs against *Plasmodium cynomolgi* malaria in rhesus monkeys. Results of these studies in subhuman primates are used in the U.S. Army antimalarial drug development program to guide the design of further animal experiments and to aid in the selection of drugs for human trials.

DESCRIPTION: These are a continuation of studies initiated in 1971, and reported with details of methodology in the SEATO Medical Research Laboratory Annual Reports for 1972–1974. These studies include an evaluation of blood schizontocidal activity of candidate compounds in rhesus monkeys inoculated intravenously with 5×10^8 parasitized erythrocytes obtained from donor monkeys infected with *Plasmodium cynomolgi* strain B. Test drugs are administered daily by stomach tube for seven days beginning four days after the parasite inoculation. Suppression of parasitemia is indicative of blood schizonticidal activity, and monkeys in which parasitemia fails to reappear for one month after splenectomy at 30 days are considered cured. Drug tolerance studies, in which a minimum tolerated dose is established and major toxic effects characterized, are also conducted in rhesus monkeys.

This year pilot studies to establish a rhesus monkey test system to evaluate candidate compounds for causal prophylactic or radical curative activity have been initiated, using methodology patterned after that developed in other laboratories. Techniques for infecting *Anopheles balabacensis* with *Plasmodium cynomolgi* strain B and for reproducibly infecting rhesus monkeys with infective sporozoites are being refined.

RESULTS:

Blood Schizonticidal Tests: This year 19 experimental antimalarial drugs were evaluated for blood schizonticidal activity. Minimum curative doses are indicated in Table 1. A number of the newer 8–aminoquinolines, particularly WR 182232 have greater activity against blood schizonts and are less toxic than primaquine. Two novel compounds, WR 194965 and WR 204165 have excellent antimalarial activity. A study performed with a formulated mixture of sulfadiazine and WR 158122 (a 2,4–diaminoquinazoline) suggests that these compounds are synergistic in combination. Minimum curative doses for the individual components of the mixture were 100 and 1.0 mg/kg respectively.

Tolerance Tests: Drug toxicity studies were conducted with six compounds. Results are summarized in Table 2. Hepatic toxicity has been encountered in two 8–aminoquinolines (WR 181023 and WR 182232), and in two benzamidines (WR 4931 and WR 199385).

Sporozoite Induced Tests: The suitability of *Anopheles balabacensis* for mass production of infective *P. cynomolgi* strain B sporozoites has been established. In a series of preliminary experiments, this mosquito has been demonstrated to be hardy and an aggressive feeder on rhesus monkeys. Engorgement rates have regularly been above 85%, and 90% or more of the engorged mosquitoes have developed moderate to heavy sporozoite infections. Baseline studies with primaquine are in progress, and the testing of experimental compounds in prophylactic and radical curative regimens in the sporozoite–induced system will be initiated in the coming year.

SUMMARY: Six antimalarial compounds have been evaluated for toxicity in rhesus monkeys, and 19 for blood schizonticidal activity against *P. cynomolgi* strain B. Sporozoite-induced test systems are being developed to permit the evaluation of causal prophylactic and radical curative activity of antimalarial drugs in rhesus monkeys.

Table 1. Summary of Blood Schizonticidal Tests in Rhesus Monkeys

Type of Compound	WRAIR Drug Number	Minimum Curative Dose (mg/kg/day)
4-Aminoquinoline	1544	10.0
8-Aminoquinoline	2975 (Primaquine)	NC[1] (31.6)
	6020	NC[1] (100.0)
	181023 (lot 1)	10.0
	181023 (lot 2)	31.6
	182232	3.16
	182234	10.0
4-Quinolinemethanol	184806	10.0
9-Phenanthrene-methanol	181018	31.6
4-Pyridinemethanol	182231	10.0
Sulfonamide	4629 (Sulfalene)	100.0
	4873	NC[1] (100.0)
Miscellaneous	5473	3.16
	5949 (Trimethoprim)	100.0
	25979 (Nitroguanil Hydrochloride)	31.6
	190830	100.0
	194965	3.16
	204165	3.16
Combination Study	7557 (Sulfadiazine) 10 : 1 158122	1.0:0.1

[1] Not curative. The compound had suppressive activity, but did not cure at the maximum dose tested. The maximum tested dose is indicated in parentheses.

Table 2. Summary of Drug Tolerance Studies in Rhesus Monkeys

Compound Number	Maximum Tolerated Dose (mg/kg/day)	Principle Toxic Effect
WR 4931	< 3.16 (I.M.)	Liver Damage
WR 172435	< 316 (Oral)	Emesis
WR 181023	< 10 (Oral)	Liver Damage
WR 182232	< 31.6 (Oral)	Liver Damage
WR 184806	10 (Oral)	Emesis
WR 199385	< 3.16 (I.M.)	Liver Damage

Relationship Between Erythrocytic Adenosine Triphosphate (ATP) Level and Human Malaria

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OBJECTIVE: To establish a quantitative assay of ATP in human erythrocytes for the determination of normal erythrocytic ATP levels in a Thai population and to determine its relationship to malaria infection.

BACKGROUND: It is known that there is considerable variation in the levels of erythrocytic ATP between individuals in a population, and that this level is constant in healthy individuals (1, 2). Comparative studies in American Negroes and Caucasians indicated the existence of different mean quantities of erythrocytic ATP between these two groups. Since the gene pool of the American Negro is derived from an African Negro stock exposed to malaria for many generations, the lower mean levels of ATP in this group suggests selection pressure caused by malaria. Further studies revealed that there is a strong positive correlation between the erythrocytic level of ATP and *P. falciparum* parasitemia (3). In human as well as simian infections, high ATP levels were directly associated with relatively high peak parasite counts (4). It has been suggested that the protective mechanism against malaria infection may result from the following:

a. Erythrocytic ATP levels of the host play an important role in supporting the initial increase of parasitemia. With the lower level of ATP, a retardation of the primary increase in parasitemia is seen, resulting in a less severe clinical course of infection.

b. The role of ATP in maintaining metabolism and viability of living cells indicates that erythrocytes with low ATP levels are less capable of maintaining their viability. This would result in the inability of the intraerythrocytic asexual parasites to develop completely, and the parasitized erythrocytes may rupture prematurely.

The purpose of this study is to investigate erythrocytic ATP levels in Thai populations continuously exposed to malaria infection, and to compare them with populations from nonendemic areas.

DESCRIPTION: A technique for quantitative assay of erythrocytic ATP utilizing a firefly luminescence method described by Stanley and Williams (5) was utilized. A calibration curve of ATP was obtained by adding an aliquot of fresh extract of desiccated firefly lanterns to various known concentrations of ATP in phosphate buffer pH 7.4. The resulting light pulses were counted in the liquid scintillation spectrometer.

In most experiments, heparinized blood was used for the quantitative assay. Immediately after venipuncture, the blood was precipitated with trichloroacetic acid and maintained at −70°C for assay of ATP. It was found that delaying precipitation of the blood resulted in a significant decrease in ATP levels (6). To prevent this loss, ACD solution at pH 5 was used as an anticoagulant (7) when immediate processing of blood specimens was not possible. One milliliter of blood was added to EDTA, mixed, and kept at 40°C (wet ice) for cyanmethemoglobin determination.

Populations from Bangkok and Lumpoon representing a nonendemic group were compared with an endemic group from Chonburi and Prachinburi. In addition to these two groups, another group of newborn infants from Women's Hospital in Bangkok was included in this study as a control.

RESULTS: As shown in Table 1 the mean erythrocytic ATP level of the nonendemic group was 3.78 micromoles/gram hemoglobin, and that of the endemic group was 3.47 micromoles/gram. In newborn infants, the ATP level was found to be higher than that of adults. This finding in newborn infants agreed with that reported by Gross et al (8), although the assay methods utilized were different. With the firefly luminescence technique, the results were found to be slightly higher than those produced by the hexokinase, G–6–PD technique. There was no significant variation of the mean levels of erythrocytic ATP in either group. Birthplaces and residential areas were used for comparison in this study; however, both groups shared a common gene pool. It appears that the quantitative level of erythrocytic ATP is under genetic control of a multifactorial type and this finding is more suggestive of a genetic control than an environmental control.

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Table 1. A Comparison of Erythrocytic ATP Levels in Thai Populations Residing in Nonendemic and Endemic Malarial Areas

Subject Population	No. Case	Micromole ATP/gm Hb		Hb gm/100 ml.	
		Range	Mean	Range	Mean
Newborn					
Bangkok	120	3.2 – 7.3	4.83	11.2 – 18.5	14.96
Nonendemic Areas					
Bangkok	98	2.62 – 5.8	3.77 } 3.78	8.6 – 17.5	14.4
Lumpoon	84	2.66 – 5.0	3.79 }	7.6 – 17.3	13.7
Endemic Areas					
Chonburi	29	2.19 – 4.3	3.06 } 3.47	8.7 – 14.3	10.81
Prachinburi	198	2.91 – 5.2	3.89 }	6.6 – 17.3	13.64

Studies of New Experimental Intermediate and Paratenic Hosts and Modes of Transmission of *Gnathostoma spinigerum*

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OBJECTIVE : To attempt to identify new experimental host animals susceptible to *Gnathostoma spinigerum* as reported in the SEATO Medical Laboratory Annual Report April 1972 to March 1973.

BACKGROUND : Some species of crustacean, namely fresh water crabs, shrimps and prawns, are occasionally eaten raw or insufficiently cooked by man. Experimentally it has been shown that fresh water crabs (*Paratelphusa sexpunctatum* and *Potamon smithanus*) could be infected with *G. spinigerum* advanced third stage larvae; therefore, they may be considered as a potential source of natural infection to man (1). After experimentally feeding shrimps and prawns (*Macrobrachium rosembergi* De Mann, and *M. mirabile* Kemp) with gnathostome larvae, it appeared that they could not be infected; however, only a few shrimps and prawns were utilized in the study (2). This study was expanded to include all larval stages of *G. spinigerum* and larger numbers of shrimps and prawns.

DESCRIPTION : In addition to the prawns obtained from sources appearing in the above report, six more living adult prawns (*M. rosembergi*) were obtained from a restaurant in Pathum Thani near Bangkok. These prawns were caught from the Chao Phya River a few kilometers north of Bangkok. Before initiating this study the prawns were maintained in fresh-water aquaria for four to five weeks for acclimation

Table 1. Results of Feeding *Gnathostoma spinigerum* larvae to Fresh-water Shrimp and Prawn.

Method of Feeding	Recipients		No. Larvae Used			Remarks
	Shrimp	Prawn	Newly Hatched or First Stage	Fully Developed in Cyclops	Advanced Third-stage from Mice	
Natural	100	—	20000	—	—	Autopsies negative after 1–35 days
Natural	100	—	—	1000	—	Autopsies negative after 1–81 days
Artificial	—	3	—	250	—	Autopsies negative after 31 and 57 days
Natural	—	8	—	—	215	Autopsies negative after 4–42 days
Artificial	—	5	—	—	48	Autopsies negative after 10–30 days
None	100	12	—	—	—	Control–autopsies negative

to the laboratory environment. From a total of 28 adult prawns obtained from the experimental farm of the Department of Fisheries and those from the Pathum Thani restaurant, 12 were autopsied for the presence of gnathostomes. The remaining 16 were experimentally fed with varying numbers of larvae fully developed in cyclops, and also advanced third–stage larvae from infected mice.

Two methods were used for feeding gnathostome larvae to prawns. Natural feeding was accomplished by presenting the prawns with both cyclops and minced mouse tissue containing known numbers of infective larvae. After feeding, the prawns were observed visually every one to two hours until all cyclops and mouse tissue were consumed. This usually occurred in less than six hours. Artificial feeding was performed by use of a polyethylene tube attached to a needle and a 1.0 ml syringe containing a known number of larvae in a few drops of fresh water. The tube was easily inserted into the mouth of the prawns and the larvae were injected. Autopsies were performed from 4 to 57 days after feeding.

Shrimp proved to be too small for successful artificial feeding; therefore, only natural feeding was used.

PROGRESS: A review of the experimental feeding of G. *spinigerum* larvae to fresh water shrimps and prawns is presented in Table 1.

SUMMARY: Fresh water shrimps and prawns (*Macrobrachium rosembergi*, De Mann) were not infected by feeding on larvae of G. *spinigerum*. The evidence does not indicate that these crustaceans can act as intermediate or paratenic hosts for G. *spinigerum*.

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Chemotherapy of Gnathostomiasis

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OBJECTIVE: To continue to search for chemicals with chemotherapeutic activity against advanced third-stage larvae of *Gnathostoma spinigerum*.

BACKGROUND: These studies are a continuation of the work reported in previous years. Many antihelmintic drugs have been evaluated for possible chemotherapeutic activity against experimental *G. spinigerum* infections of mice with advanced third stage or migrating larvae. All drugs have been ineffective so far.

DESCRIPTION: Mice of the ICR strain were infected by oral administration of five advanced third-stage larvae of *G. spinigerum*. After infection for some days, the test drug or combination of drugs dissolved in distilled water was administered orally or parenterally in a predetermined regimen. Infected control mice were given distilled water orally. After completion of the treatment regimen, the mice were sacrificed at intervals and necropsied. Parasites were counted in the liver and/or body muscles and the results recorded.

The drugs tested during this reporting period were: Astiban (sodium antimony dimercapto-succinate), Lucanthone (Miracil D. or Nilodin) (1-methyl-4-diethylamino-ethylaminothioxanthone hydrochloride), Hycanthone (Etrenol) an active metabolite of Lucanthone, and Iodine in Lugol's solution.

PROGRESS: Drug screening tests on mice infected with *G. spinigerum* advanced third stage larvae gave the following results.

Astiban: This drug was administered last year in five daily oral doses of 640 mg/kg or with a single oral dose of 1920 mg/kg to gnathostome-infected mice without effect. This year an oral dose of 1920 mg/kg daily for two days was also found to be ineffective in significantly reducing the numbers of the larvae in treated mice. Therefore the drug appears to have no therapeutic effect on infected mice (Table 1).

Lucanthone: Gnathostome-infected mice were treated with two doses of 150 mg/kg/day for five days (ten doses). The results are shown in Table 2. This drug is judged to have no therapeutic value in the treatment of *G. spinigerum* infection.

Hycanthone: This drug was administered orally over a five day course using doses of 100, 200, 300, and 400 mg/kg. The results are shown in Table 3. There was no significant reduction in the number of gnathostome larvae in the treated mice. Therefore Hycanthone is considered to have no therapeutic effect on the infection.

Lugol's solution: The prescription of the solution was Iodine, two grams; Potassium Iodide, four grams; and purified distilled water, 100 ml. An *in vitro* experiment of various dilutions of the solution (1:1000 to 1:20,000 or iodine solution equivalent of 1:500 to 1:10,000) on living *G. spinigerum* advanced third-stage larvae obtained from the experimentally infected mice caused the death of the larvae in ten minutes to four days compared with the control in distilled water where the larvae lived for eight days.

The screening tests on infected mice (average body weight of 25 grams per mouse) were done by oral administration of various doses of the solution containing iodine 40, 200, and 400 mg/kg (or iodine solutions in mice of 1:625, 1:125, 1:60) twice daily for five days. The results showed no therapeutic value on the infection (Table 4), and all five infected mice who received 400 mg/kg died of toxicity about six hours after the administration of the last dose.

The drug was also given by subcutaneous injection of Lugol's solution using a dose of 20 mg. iodine/kg body weight of the infected mouse or equivalent to about 1:1250 solution of iodine in the mouse body. The result is shown in Table 5. This dose of the drug by subcutaneous administration is judged ineffective.

SUMMARY: Oral administration of Astiban, Lucanthone, Hycanthone and Iodine in Lugol's solution and subcutaneous injection of Iodine in Lugol's solution were ineffective in the chemotherapy of *Gnathostoma* spinigerum in experimentally infected mice. Further investigation on Iodine in Lugol's solution given by subcutaneous injection is in progress and the combined therapy with Astiban and Ambilar has shown a modest chemotherapeutic effect and will be investigated further (1).

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Table 1. Treatment of *Gnathostoma spinigerum* Infected Mice with Astiban Oral Administration

Astiban Drug Dose (mg/kg/day)	No. of Infected Mice Treated	Third–stage Larvae Found	Time of Necropsy (Days)*
1920**	30	90	20
Control	30	93	19

* Days after administration of last drug dose
** For two days

Table 2. Treatment of *Gnathastoma spinigerum* Infected Mice with Lucanthone Oral Administration*

Lucanthone Drug Dose (mg/kg/day)	No. of Infected Mice Treated	Third–stage Larvae Found	Time of Necropsy (Days)**
150	10	31	24
Control	5	16	24

* Two doses per day for five days
** Days after administration of last drug dose

Table 3. Treatment of *Gnathostoma spinigerum* Infected Mice with Hycanthone Oral Administration*

Hycanthone Drug Dose (mg/kg/day)	No. of Infected Mice Treated	Third–stage Larvae Found	Time of Necrospy (Days)**
100	15	45	4–27
Control	15	46	14–28
200	13	42	10–27
Control	15	46	14–28
300	15	45	4–40
Control	15	44	33–40
400	14	40	1–40
Control	15	44	33–40

* One dose per day for five days
** Days after last dose of drug

Table 4. Treatment of *Gnathostoma spinigerum* Infected Mice by Oral Administration of Iodine in Lugol's Solution*

Iodine Drug Dose (mg/kg/day)	No. of Infected Mice Treated	Third–stage Larvae Found	Time of Necropsy (Days)**
40	5	19	7
Control	5	20	3–7
200	5	16	17
Control	5	17	17
400	5	17	All mice died six hrs. after last dose

* Two doses per day for five days
** Days after last dose of drug

Table 5. Treatment of *Gnathostoma spinigerum* Infected Mice by Subcutaneous Injection in Iodine in Lugol's Solution*

Iodine Drug Dose (mg/kg/day)	No. of Infected Mice Treated	Third–stage Larvae Found	Time of Necropsy (Days)**
20	10	30	17
Control	5	16	17

* One dose daily for five days
** Days after the last dose

Toxoplasmic Lymphadenitis

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OBJECTIVE: To study the incidence of toxoplasmic lymphadenitis in patients with acute or chronic posterior cervical lymphadenopathy.

BACKGROUND: A human case of congenital or acquired toxoplasmosis has not been reported in Thailand. Toxoplasmic lymphadenitis is said to be the most common manifestation of the infection in man.

DESCRIPTION: Patients with enlargement of posterior cervical lymph nodes, attending Pramongkutklao Hospital in the outpatient department were picked for the study. Their sera were collected for a hemagglutination test. Any patient whose blood was found to contain the antibody would be asked to cooperate in having a lymph node biopsy done and an isolation attempt made.

PROGRESS: Toxoplasma antibody at the titer 1:640 was found in only one of 32 sera collected during November 1974—March 1975. An attempt has been made to get in touch with the patient, a resident of Korat.

MISCELLANEOUS

An Epizootic of Tropical Canine Pancytopenia in Thailand

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OBJECTIVE: To study the epizootology of Tropical Canine Pancytopenia in a population of military working dogs, and to evaluate the efficacy of currently recommended prophylactic and therapeutic measures in a natural outbreak.

BACKGROUND: Tropical Canine Pancytopenia (TCP) is a tick-transmitted infectious disease of dogs caused by the rickettsia-like organism *Ehrlichia canis*. Infected dogs may be almost asymptomatic, or they may develop a frequently fatal syndrome characterized by fever, anemia, leukopenia, thrombocytopenia, and hemorrhage. The fatal syndrome has been observed most frequently in German Shepherds. Outbreaks of TCP have occurred in many tropical and sub-tropical countries, but until now the disease has not been recognized in Thailand. The present report describes an epizootic of TCP among military working dogs at the War Dog Training Center, Pakchong, Korat Province, Thailand, 185 km northeast of Bangkok.

DESCRIPTION: The War Dog Training Center is a modern facility established by the Thai Armed Forces to breed, train, and issue working dogs to all military services. The population of dogs at the Center averaged about 175 during the period of the study. The Center also supports an additional 125-150 dogs at remote stations. The composition of the dog population at the Center is constantly changing as dogs are issued to and return from remote stations. Most working dogs return to the Center annually for retraining; dogs also return for treatment of serious disease problems, since veterinary care is not generally available except at the Center. Dogs at the Center are maintained in individual pens in several groups of screened or unscreened buildings which are separated by distances as great as several hundred meters. These groups of buildings are designated as the breeding area, the young adult area, the training area, the working dog area, and the hospital area. There is limited direct daily contact between groups, although dogs are periodically moved from one functional area to another as needs of training and utilization dictate. Contact is also possible in the hospital area which services dogs from all areas and from outside the Center. In addition, indirect contact is possible in common exercise, training and working areas. The common brown dog tick (*Rhipicephalus sanguineus*) has been collected from dogs and kennels in all areas. No other species of tick has been found.

TCP was first suspected in March, 1974, among a group of seven German Shepherds working at a military base in Lopburi, 133 km north of Bangkok. Within a two month period, three of these dogs died after episodes of epistaxis. The four surviving dogs, which were in poor condition, were transported to Pakchong and placed in the hospital for observation and treatment. It is believed that these dogs may have initiated the epizootic at Pakchong, although a retrospective analysis of clinical records suggests an increase in the prevalence of febrile episodes among dogs at Pakchong as early as January, 1974. No dogs were imported to the Center from outside Thailand during this time nor during the year prior to the recognition of TCP at Pakchong.

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The progress of the epizootic of TCP has been followed by clinical, pathological, and laboratory studies. A clinical record is maintained on each dog, which contains an indication of clinical symptoms observed and treatments administered whenever a dog is brought to the hospital. Complete physical examinations are performed at regular intervals, including surveillance and treatment for heartworm and intestinal parasites. All dogs are regularly vaccinated for canine distemper, canine hepatitis, leptospirosis, and rabies.

A program to control the epizootic was developed based upon the experiences in Vietnam (1, 2) and upon laboratory studies conducted at the Walter Reed Army Institute of Research in subsequent years (3, 4). Serologic identification of infected dogs was the cornerstone of the control effort (5). The basic elements of the control program were as follows:

1. Identification of infected dogs by serologic testing, clinical signs, and laboratory studies.

2. Treatment of infected or suspect dogs with tetracycline hydrochloride orally 30 mg/lb/day for 14 days. Supportive therapy was utilized as appropriate in severe clinical cases.

3. Prevention of infection or re-infection by continuous daily oral administration of approximately 3 mg/lb/day of tetracycline hydrochloride. (A single 250 mg capsule was opened, and the powder lightly mixed with the pre-weighed food in each dog feeding pan).

4. Elimination of ticks by regular spraying of kennels and dipping of dogs with insecticide.

5. Isolation and treatment of newly introduced dogs.

The laboratory procedures utilized to identify TCP-infected dogs for treatment included serology, hematocrit, total and differential leukocyte counts, and serum protein electrophoresis. The serological method utilized was the indirect immunofluorescent test (5). At each bleeding 10 ml of venous blood was collected. Two to three milliliters were placed in tubes with EDTA for hematologic studies; the remainder was allowed to clot and serum collected for serology and serum protein determinations. For serologic screening, a 1:10 serum dilution was used, and results were reported as "positive" or "negative".

Blood samples were collected from all dogs at Pakchong (except puppies less than six months of age) at three month intervals. During the intervals between quarterly bleedings some additional dogs were bled, including new arrivals missed at the previous bleeding. On occasion, sera were collected at other military bases, but the bleeding of dogs at remote stations could not be comprehensive. Quarterly bleedings were performed on 4 June, 4 September and 18 December, 1974, and on 4 March 1975. In addition, all dogs at Pakchong were bled on 25 July 1974, just before initiation of the tetracycline treatment program. A total of 316 dogs were studied between June 1974 and March 1975. This included 287 German Shepherds, 10 Doberman Pinschers, 9 Labrador Retrievers and 10 Labrador-Shepherd cross-breeds.

Individual dogs were given a complete 14 day therapeutic course of tetracycline for any of the following reasons:

1. Suspicious clinical signs
   a) Unexplained fever
   b) Anemia (hematocrit less than 39)
   c) Leukopenia (WBC less than 6000)
   d) Bleeding (epistaxis, hematuria)
   e) High serum gamma globulin (> 2.5 gm%)
2. Serologically positive for TCP
3. Known or suspected exposure to TCP
4. Admitted to the Center with unknown prior history

In addition, on 26 July, all dogs at the Center older than six months of age (172 dogs) were placed on a 14 day therapeutic course of tetracycline. This was done with the knowledge that 49 per cent of the dogs had been serologically positive on 4 June, and with the suspicion than numerous additional dogs were incubating the disease (31 dogs developed fevers between 4 June and 25 July). At the completion of treatment, all dogs were continued on prophylactic levels of tetracycline daily until 9 September. After a 60 day interruption, tetracycline prophylaxis was again reinstituted on 9 November, and has been maintained until the present time. If at any time a previously treated dog converted serologically, or showed any of the suspicious clinical signs listed above, it was re-treated with tetracycline therapeutically.

PROGRESS: The existence of TCP in Thailand has been demonstrated conclusively for the first time. The evidence for the disease includes the observation of characteristic clinical, hematological and pathological signs among a group of Thai Military Working Dogs, and the confirmation of the disease serologically and by direct observation of morulae of *Ehrlichia canis* in tissue macrophages.

In April 1974, Krisda, a one year old, male German Shepherd, was transported from Pakchong to SEATO Laboratory for observation and diagnostic studies. This dog had a history of recurrent fever (102°F–104°F), inappetence, and progressive weight loss. On admission, the hematocrit was 35; leukocyte count 5100 per cmm; fecal specimen negative for ova and parasites. The serum was serologically positive for TCP. During a month-long period of observation, rectal temperature was consistently elevated (103°F–105.8°F), the appetite was poor, and the dog became progressively more debilitated, lost weight, and developed pressure ulcers of the skin of the abdomen and over bony prominences. The hematocrit fell to 17; leukocyte counts ranged from 6,600 to 30,000 per cmm. No chemotherapy was given. The dog was ultimately euthanized. At necropsy a moderate, multifocal bronchopneumonia was observed. Multifocal plasma cell infiltration was prominent in lymph nodes, in portal areas and around central veins of the liver, and interstitially in the kidneys. Lymphocytic and plasmacytic vasculitis, occasionally with minimal perivascular hemorrhage, was observed in the brain. Typical inclusions or "morulae" of *Ehrlichia canis* were observed in macrophages in giemsa-stained impression smears obtained from cut surfaces of lung at necropsy (4).

Between early April and 25 July, many dogs exhibited clinical or hematological abnormalities suggesting TCP. The number of dogs treated with tetracycline because of fever, anemia or epistaxis during these months is indicated in Table 1. During this period of rapid disease transmission, 47 dogs were treated with tetracycline on the basis of clinical signs without knowledge of their serological status (results of 4 June serology were not known until after 25 July). Only two of these dogs were later found to be serologically negative, and thus 97% of these treatments were appropriate. On the other hand, of the 86 dogs serologically positive on 4 June, only 45 (52%) were selected for treatment on the basis of clinical signs or hematology.

After the mass treatment of dogs on 25 July, a dramatic improvement in the general condition of dogs was noted. Thin dogs gained weight, work performance improved, breeding performance improved, and the number of dogs hospitalized was reduced. We continued to initiate tetracycline therapy in dogs developing fever, anemia or epistaxis, as indicated in Table 2. Thirty-eight dogs were treated for symptoms between 26 July 1974 and 31 March 1975. Nineteen of these courses of treatment (68%) were apparently inappropriate, since they were administered to dogs which were serologically negative both before and after treatment.

Compared to the experience in Vietnam, the severity of the clinical disease at Pakchong was mild. Between April 1974 and March 1975, only 19 dogs died at Pakchong. Six of these deaths were from causes other than TCP (accident, heartworm-2, drug toxicity, cystic calculi, heat exhaustion). Three dogs died of unknown causes. In ten dogs the history, clinical findings, laboratory studies, and gross necropsy findings suggested that TCP was the primary or contributing cause of death. Histopathologic confirmation of TCP was possible in only two cases. Only two of the TCP deaths occurred after 26 July. Both of these dogs were returned to Pakchong from remote sites in moribund condition.

Tetracycline therapy had been instituted in only one of the ten dogs which died of TCP. In this case, the dog was exhibiting epistaxis, the hematocrit was 14, and the dog died three days after initiation of therapy. In all other severe cases tetracycline therapy caused remission of symptoms. Nine dogs with epistaxis and 13 dogs with hematocrits of 15–25 have been successfully treated.

Severe symptoms of TCP were observed only in German Shepherds, although 7 of 9 Labradors, 5 of 10 Dobermans, and 1 of 10 Shepherd–Labrador cross–breeds were serologically positive. Two of the serologically positive Labradors had febrile episodes, and two had low hematocrits (28 and 31); they were otherwise asymptomatic. Three Dobermans were febrile, but exhibited no abnormal hematologic signs prior to treatment. The Shepherd–Labrador cross–breed dog was asymptomatic.

The results of serologic studies are presented in Table 3. The progress of the epizootic can be followed both before the institution of control measures on 26 July, and during the period of control.

Initially, on 4 June, 86 serologically positive dogs (49% of those at the Center at that time) were identified. By 25 July, six weeks later, 30 additional dogs had converted to positive (33% of the 90 susceptible), and 17 more dogs entering the Center from remote sites were identified as positive. After the dogs were placed on tetracycline therapy and prophylaxis, the rate at which dogs converted to positive markedly decreased. Between July and October, only 13 converted, and it is likely that the infections producing these conversions were mainly acquired before 25 July. Experimentally infected dogs do not develop detectable titers for 11–28 days (5). The three dogs converting after October spent a portion of their time away from Pakchong, and were not maintained on tetracycline prophylaxis.

All known serologically positive dogs have been treated with tetracycline, and many are now becoming serologically negative. The large number of dogs converting to negative between December 1974 and March 1975 largely reflect the treatment given in June and July. Of the 69 dogs remaining serologically positive, only 16 were bled at the Center in March 1975. The remaining 53 dogs were at remote stations, and although they were serologically positive at their last bleeding, current serology is not available on the majority of them.

Curiously, the prevalence of serologically positive dogs entering the Center from remote sites is decreasing, although tetracycline has not been administered prophylactically except at Pakchong. Apparently the elimination of infected dogs at Pakchong, and improved tick control at remote sites are having a beneficial effect.

Serum protein electrophoresis has been investigated as a diagnostic procedure to supplement serologic and hematologic findings. An elevation of serum globulins, particularly beta and gamma globulins, has been observed in experimentally infected dogs (Huxsoll, personal communication).

Some, but not all, infected dogs at Pakchong have exhibited increased gamma globulins. In most cases this was also reflected in a reduced A/G ratio. Comparison of the mean values for serologically positive and serologically negative dogs revealed no significant differences; however, individual dogs among the infected groups did have gamma globulin values which differed significantly from values observed in serologically negative dogs. Among serologically positive dogs prior to treatment, 30 of 85 (35%) had significantly elevated gamma globulins on 4 June, and 13 of 38 (34%) had elevations on 25 July. Abnormal gamma globulin levels returned to normal after drug treatment more rapidly than the dogs converted to negative serologically. By 25 July, only 6 of 24 treated dogs (25%) had elevated gamma globulins, and by 4 September only 4 of 80 (5%) still had abnormal values.

The ability to identify infected dogs serologically has been indispensible to the control effort. The treatment of dogs using clinical or hematologic criteria has proven unsatisfactory. Many dogs have been treated unnecessarily using symptomatic criteria, and, more importantly, many asymptomatic carriers would have been overlooked.

An important limitation of the serologic screening method is its inability to identify re-infections or failed treatments. Dogs are susceptible to re-infection even though they have serologically demonstrable antibody to *Ehrlichia canis* (3). After treatment, for as long as the dog remains serologically positive, only clinical and hematological observations serve to identify re-appearance of active infection. As of March 1975, 69 treated dogs remained serologically positive. It is entirely possible that some of these dogs are harboring the organism. Hopefully, control of ticks and the continuation of prophylactic tetracycline will prevent transmission of the organism until such time as any carriers among this group can be identified.

Efforts to control the epizootic in Thai military working dogs are continuing. Tetracycline is still being administered prophylactically, and dogs which exhibit suspicious clinical or hematologic signs, or which convert serologically, are being treated with tetracycline. While many dogs have become serologically negative, the efficacy of the combined therapeutic and prophylactic administration of tetracycline cannot be evaluated until the infective status of the remaining 69 serologically positive dogs has been resolved. Efforts to control ticks are being maintained.

SUMMARY: An epizootic of Tropical Canine Pancytopenia (TCP) has been studied in a population of 316 Thai Military Working Dogs. To date, 161 cases have been identified serologically, of which 54 were clinically or hematologically apparent. The prevalence of severe clinical symptomatology was low. Epistaxis was observed in only 9 dogs (2.8%), and only 10 dogs died (3.2%).

A control program including tick control, serologic identification and treatment of carriers, and tetracycline prophylaxis has been instituted. Administration of tetracycline 30 mg/kg/day for 14 days has produced clinical remission of symptoms in all but one of the severely ill dogs. Serologic remission has been observed in all but 69 dogs to date. Early intervention with tetracycline is probably largely responsible for the mildness of the epizootic, but the possibility that the organism was less virulent, or the dogs more resistant than in the Vietnam epizootic, cannot be ruled out.

Clinical, hematological, and serological surveillance is continuing.

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Table 1. Major Clinical Signs of TCP Observed at Pakchong During June – July 1974

Clinical Signs	Number of Dogs Exhibiting Signs	
	Serologically Positive	Serologically Negative
Fever (a)	31	2
Anemia (b)	8	0
Epistaxis (c)	6	0
Total	45	2

(a) Rectal temperature above 103°F. Depression and inappetence were frequently associated with the fever, but diarrhea or vomiting were seldom observed. None of these dogs exhibited anemia, leukopenia or epistaxis.

(b) Hematocrit less than 39. None of these dogs exhibited epistaxis; some were leukopenic, and most were febrile intermittently.

(c) These dogs were also anemic, leukopenic and febrile.

Table 2. Major Clinical Signs of TCP Observed at Pakchong Between August 1974 and March 1975

Clinical Signs (a)	Number of Dogs Exhibiting Signs	
	Serologically Positive	Serologically Negative
Fever	2	0
Anemia	7	19
Epistaxis	0	0
Total	9	19

(a) See footnote for Table 1.

Table 3. Results of Serologic Studies at Pakchong – June 1974 to March 1975

Date	Cumulative Number of Dogs Studied (a)	Serologically Positive Dogs			Serologically Negative Dogs			Cumulative Known Positive Dogs	Cumulative Known Negative Dogs	Percent Positive
		Converted To Positive	Added To Study	Total New Positive	Converted To Negative	Added To Study	Total New Negative			
4 Jun 74	176	—	86	86	—	90	90	86	90	49 %
25 Jul 74	200	30	17	47	8	13	21	120	80	60 %
Sep-Oct 74	242	13	12	25	6	33	39	136	106	56 %
Dec 74–Jan75	294	3	2	5	45	53	98	93	201	32 %
Mar 75	301	0	1	1	23	9	32	69	232	23 %

(a) Dead Dogs Excluded

Prediction of Illicit Drug Use by United States Servicemen

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OBJECTIVE: For a description of the objectives of this study see the SEATO Annual Progress Report, March 1972.

PROGRESS: Interviews, at four-month intervals, of 425 soldiers in Thailand have been completed. We were able to obtain complete information on 345 of these men. The remaining individuals were interviewed only one or two times. Twenty-five additional individuals left Thailand within four months after arriving and were therefore not interviewed at all.

Data derived from these interviews have been collated and coded for automatic data processing. Analyses of these data are currently being conducted. These will be used to determine: a) the predictive validity of the questionnaire instrument, b) variables which relate to development of risk for drug use and c) variables which interfere with the ability of the instrument to predict drug use.

In addition to behavioral information concerning drug abuse, we have drawn bloods from these individuals and obtained medical histories. Review of medical and laboratory records was also conducted. This will provide unique data on development of inapparent disease in this population during a tour in Thailand.

SUMMARY: Interviews have been completed on 425 soldiers during their first year in Thailand. Drug use classifications based on the clinical interview and the questionnaire instrument will be compared to evaluate the predictive validity of the questionnaire. The analyses will focus on variables contributing to risk for drug use. Information concerning development of inapparent infection will provide unique epidemiological data on this population.

A Behavioral Survey of Thai Prostitutes

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OBJECTIVE: a) To collect behavioral information from a prostitute population concerning attitudes and knowledge about venereal disease (VD), b) to correlate these with certain concurrently collected laboratory data and c) to determine what difference exists between these variables for women soliciting Americans and those women who do not have contact with Americans.

BACKGROUND: The prevalence of venereal disease among American troops seems to many observers to be too high. Considerable man–hours and money are spent on treatment of VD in American troops and on programs for the control of VD among prostitute populations. An evaluation of the variables under study will provide information on the effectiveness of US military sponsored venereal disease control programs directed at these prostitute populations.

DESCRIPTION: Laboratory and interview data were collected from 520 women. Four hundred and forty –four of these solicit US military personnel and 76 solicit only from the local national (Thai) population. Most women were selected from those attending VD clinics, but 20% were selected from other sources in order to gain information most representative of the entire population.

PROGRESS: Data are currently being analyzed. It is anticipated that analysis and write–up will be completed by July 1975.

SUMMARY: Laboratory and interview data were collected from a total of 520 Thai prostitutes. This will provide unique behavioral and laboratory information on VD in this population and effectiveness of VD control programs. Data analysis and write–up are currently being conducted.

Mosquito Fauna of Thailand

Principal Investigators:

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OBJECTIVE: To collect, identify, catalogue and redescribe the mosquito species of Thailand. Information is also being gathered on the distribution, larval habitats and other aspects of the bionomics of various species. The eventual goal is the production of monographs on the mosquitoes of the area, together with keys, handbooks and other identification aids, for use of workers in public health and associated fields.

DESCRIPTION: Mosquitoes are collected from many areas of Thailand in connection with various studies on malaria and other arthropod–borne diseases. Additional collections of a specialized nature are made to obtain a correlated series of larvae, pupae and adults for illustration and taxonomic studies. The majority of this material is shipped to the Smithsonian Institution for study by specialists in the Southeast Asia Mosquito Project (SEAMP).

PROGRESS: During this year 887 collections of mosquitoes were made in Lampang, Chiangmai and Kanchanaburi provinces. These collections resulted in 7,300 pinned adults, 6,190 slide mounts of larvae, larval and pupal skins and 20 slide mounts of terminalia. Progeny rearings of nine *Aedes (Finlaya) niveus* group mosquitoes from filariasis study sites in Kanchanaburi province yielded a total of 190 pinned adults, 182 slide mounts of larvae, larval and pupal skins.

Culex: An attempt to collect all species of the *Culex (Culex) vishnui* subgroup of Thailand is nearing completion. *Culex barraudi* and *Culex whitei*, rare species of this group, were collected from seepage pools, ground pools, wells and paddy fields in Chiangmai and Lampang provinces. Collections of all stages of *Culex alienus*, *C. annulus*, *C. perplexus*, *C. pseudovishnui* and *C. tritaeniorhynchus* were obtained during the previous period.

Aedes: Work on the important *niveus* complex of species in *Aedes (Finlaya)* has been concentrated mainly at Sangkhlaburi in Kanchanaburi province. *Aedes niveoides* seemed to be one of the most abundant species in this area. Another three unidentified species were obtained from bamboo cup collections.

Ten species of *Aedes (Stegomyia)* were collected during filariasis studies in Sangkhlaburi district. The immature stages of *A. albopictus* have been collected in association with those of *A. pseudalbopictus*.

Heizmannia: Approximately nine species of this genus were collected at Sangkhlaburi. *H. reidi*, *H. mattinglyi*, *H. covelli* females and an unidentified male were collected and reared from immature stages. *Heizmannia mattinglyi* which is known only from the adult female was collected in association with *H. covelli*. Associated larval and pupal skins of both these species are indistinguishable, but the male terminalia are typical of *H. covelli*.

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Pathogens of Medically Important Mosquitoes of Thailand

Principal Investigator: Stephen C. Hembree, CPT. MSC

Associate Investigator: Douglas J. Gould, Ph.D.

OBJECTIVE: To determine the kinds of insect pathogens present in medically important species of mosquitoes in Thailand and to elucidate the biology of selected pathogens sufficiently to assess their potential as biological mosquito control agents.

BACKGROUND: Successful and economically feasible biological control of certain important agricultural and forest insect pests with pathogens, used alone or in combination with other control agents, has been thoroughly documented. Several pathogens for use against agricultural pests have been approved by the Food and Drug Administration and the Environmental Protection Agency and are currently being produced commercially and used in the United States. Interest in the potential of pathogens for the control of medically important arthropods is not new, but the successful use of pathogens in agriculture and forestry, combined with widespread environmental interest, has provided impetus in recent years for more vigorous investigation of their potential value to medical entomology. The SEATO Medical Research Laboratory is an ideal location for such investigations, for in Thailand there are more than four times as many species of mosquitoes (400+) as in the United States. Among these are several species of primary international importance as disease vectors.

DESCRIPTION: During the first six months of this project the slide-mounted mosquito larvae in the taxonomic collection of the Medical Entomology Department was screened for microscopically detectable pathogens. Also, a field survey for mosquito pathogens was initiated. The survey has concentrated on *Culex pipiens quinquefasciatus* in the Bangkok-Thon Buri area. Large numbers of larvae collected at various locations were transported to the laboratory. These were visually examined for gross signs suggesting presence of pathogens, such as loss of pigmentation, presence of abnormal color, aberrant swimming behavior, and conspicuously abnormal body proportions.

Larvae displaying gross signs of pathology were segregated. Part of these abnormal larvae were examined microscopically in wet-mounts and/or as Giemsa- or hematoxylin-stained squash-smears, while the balance were prepared for paraffin sectioning and hematoxylin-eosin staining. When large collections of larvae showing grossly abnormal signs were made, a portion of the collections were reared in the laboratory and the mortality rate was recorded. Smears were made of larvae and pupae that died. Surviving pupae were allowed to develop to adults and the progeny of these examined for evidence of transovarial (vertical) transmission. Attempts to transmit pathogens by *per os* exposure of uninfected laboratory-reared larvae were also made. Survivors of these tests were reared and their progeny examined for evidence of vertical transmission.

PROGRESS: Five distinct species of fungus of genus *Coelomomyces* were found in the slide-mounted larval collection. *Culex tritaeniorhynchus* and *C. fuscacephala* were infected with apparently the same species, three different species were found in *Anopheles vagus* and one in *A. nivipes*. One nematode infection was found in *C. tritaeniorhynchus*.

Seventy-eight collections of *C. pipiens quinquefasciatus* were made at 54 locations in the Bangkok-Thon Buri area. Stained smears of larvae from 67 of the 78 collections were examined. Examination of all material collected is not complete, but the following observations have been made. Of 2073 larvae displaying grossly abnormal signs, microbial agents were found in 1971 (95%). Microbial agents were often seen within tissues or hemolymph of larvae examined in wet-mounts prior to squash-smearing. The

presence of numerous microbial contaminants on the integuments and within the alimentary canals of larvae possibly obscured infections that will become apparent with the examination of sectioned material.

Two microbial agents were present in almost all specimens showing gross signs of disease. One was a dark–staining cytoplasmic inclusion, about four microns in length, that appeared to replicate by transverse fission. This agent could be seen within cells in larvae examined in wet mounts. It has been transmitted to uninfected larvae in the laboratory. The other common agent was of minute bacilliform structure at the limit of resolution of the light microscope, somewhat less than one micron in length. This agent could be detected within the hemolymph of larvae examined in wet–mounts by its intense Brownian motion and in smears stained with Giemsa's stain at pH 7.4. Larvae containing this agent died almost without exception. The agent has been transmitted to uninfected *C. quinquefasciatus* larvae in the laboratory. Microsporidia were found in 27 of 67 (40%) collections from 15 of 54 (28%) locations surveyed. Preliminary transmission attempts have not been successful, but definitive attempts are planned.

One fungal agent has been found which presents by turning mosquito larvae orange. All transmission attempts with this agent have been unsuccessful. More material is being sought in the field.

An additional agent in the size range and with the staining characteristics of a polyhedral virus was collected from 11 of 54 (20%) locations. This agent was transmitted in the laboratory, but transmission rates were lower than those expected with a polyhedral virus and the identity of the agent remains in doubt. Further studies are underway.

DISCUSSION: Distortion of *Coelomomyces* sporangia resulting from the mounting techniques used for mosquito larval taxonomic specimens made species determination impossible. Adult forms are required for identification of insect nematode parasites, so the larval nematode found could not be identified.

Studies are underway to determine the identity and to define the biological characteristics of agents found in surveys of *Culex pipiens quinquefasciatus*. Efforts in the following areas will be required: (1) culturing of bacteria and fungi, (2) electron microscopy of suspected virus, rickettsiae, and microsporidia, (3) transmission experiments to determine optimum conditions and methods for transmission and propagation, and (4) experiments to establish host ranges. The survey for mosquito pathogens will be extended both geographically and to include other species of medical importance.

Access to the scientific literature in invertebrate pathology is essential for the identification of mosquito pathogens, and the literature is a time–saving source of methods in the propagation and study of pathogens. Therefore, a high priority will be placed on accumulating a bibliography of pertinent literature and a collection of reprints of previous reports of pathogens in mosquitoes.

Evaluation of Systemic Insecticides for Control of Trombiculid Mites

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OBJECTIVE: To determine if rodent baits treated with organophosphate insecticides are effective against larval trombiculid mites (chiggers) on wild rodents in areas of Thailand where these mites are known to carry scrub typhus.

BACKGROUND: In World War II more than 7,000 casualties were caused by scrub typhus in U.S. Forces stationed in the Western Pacific and Burma. Control measures used at that time to prevent scrub typhus included the burning of grass and the stripping of soil with bulldozers in encampments; DDT was effective for control of mosquitoes and flies, but ineffective against the scrub typhus mites. Impregnation of clothing with repellents such as dimethyl pthalate was the most effective method of personal protection against chiggers, but it did not enjoy a high degree of troop acceptance. Scrub typhus continued to be a problem during the Korean Conflict; control and prevention measures used during that period were essentially the same as used during WWII. Some of the later chlorinated hydrocarbons, such as chlordane, dieldrin and lindane, were found to be effective for area control of trombiculid mites. However, large quantities of these insecticides were required (up to 5 lbs/acre) for effective control, and because of their non-specificity these compounds destroyed innocuous and beneficial organisms. More recently, organophosphates, such as ronnel and Ruelene, have been employed as systemic insecticides to control fleas on rats and dogs and ticks on cattle with a significant degree of success. Another of these compounds, Phoxim, has been used in New Mexico to control fleas and several types of mites on wild rodents which fed on Phoxim-treated grain (1).

The principal vector of scrub typhus in Thailand is *Leptotrombidium (L.) deliense* (Walch) which parasitizes rodents, chiefly *Rattus rattus*, living in scrub forest. This mite is widely distributed throughout Thailand. During the reporting period preliminary tests of Phoxim were carried out on wild-caught *Rattus rattus* trapped near Sakaerat in Nakornratchasima province. This area is located near the site of an outbreak of scrub typhus which occurred in a unit of Thai Army personnel in 1965 (2).

DESCRIPTION: In each series of tests, wild-caught *Rattus rattus* trapped in the vicinity of Sakaerat, were inspected for the presence of larval trombiculid mites in their ears. Previous experience trapping rats in that area indicated that a high proportion of the trombiculid mites infesting *R. rattus* were *T. deliense*. Infested rats were separated into two equal groups; one was given a diet of Phoxim-treated corn and the other untreated corn. During these tests each rat was confined to a cage suspended over pans of water to catch all mites that detached from their hosts. The water was removed daily from these pens, after a visual inspection for mites on the surface, and filtered through cotton muslin which was examined under a stereoscopic microscope for chiggers. Mites found were removed and placed in vials containing a moistened plaster of Paris-charcoal base and examined daily until it was established whether death had occurred or if metamorphosis had taken place. Dead mites were placed in 70% alcohol to be later mounted and identified. Larval trombiculid mites which underwent metamorphosis were discarded and were considered to have survived. Upon conclusion of the tests all rats were sacrificed, and any chiggers still attached were removed and processed in the same manner as those recovered during the tests.

PROGRESS: Uncracked, dried corn, treated with two concentrations of Phoxim (0.24% and 0.36%), was fed to wild-caught *Rattus rattus* infected with trombiculid mites. No significant increase in mortality of mites on the rats fed treated grain was observed in either test (Tables 1, 2). In the two Phoxim trials the proportions of *T. deliense* in the mites recovered from the *R. rattus* were 53 and 70 percent for the 0.24% and 0.36% trials, respectively. In a single test with 0.36% dimethoate-treated corn, the overall mortality in mites on the animals fed treated grain was greater (24%) than among mites on the control animals (3%), but not significantly higher than was observed for either group in the Phoxim tests (Table 3). However, an unusually high proportion of the mites on the rats fed dimethoate detached on the first day of the test; 83% of the mites recovered from these animals detached on day 1, while at the same time only 22% of the mites on the control animals dropped off. If the data for the first day are excluded, the toxic effects of dimethoate appear more impressive: 89% of the mites recovered between days 2 and 10 from rats fed dimethoate treated grain were dead, while only 3% of the mites recovered during the same period from control animals died. It is probable that the dimethoate-treated grain (which had a powerful odor) had a fumigant effect on the mites, causing large numbers to detach from their host on the first day of the test before the test rats had a chance to ingest much of the treated grain. Seventy-two percent of the mites recovered from the animals in these tests were *T. deliense*.

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Table 1. Effects of 0.24% Phoxim–treated Bait on Trombiculid Mites Infecting *Rattus rattus*.

Day	TEST ANIMALS (12)		CONTROL ANIMALS (12)	
	Total No. Mites Recovered	Mite Mortality (Per cent)	Total No. Mites Recovered	Mite Mortality (Per cent)
1	285	15.1	172	23.8
2	341	27.5	323	31.8
3	71	39.4	92	15.2
4	11	27.3	25	32
5	4	100	14	50
6	10	70	46	26
7	2	50	28	0
8	1	100	5	0
9	0	—	0	—
10	2	100	0	—
11	2	50	0	—
12	2	0	0	—
13	1	0	1	100
14	667*	32.4	784*	15.6
Total	1399	28.6	1493	20.7

*Recovered after rats sacrificed

Table 2. Effects of 0.36% Phoxim–treated Bait on Trombiculid Mites Infecting *Rattus rattus*.

Day	TEST ANIMALS (13)		CONTROL ANIMALS (13)	
	Total No. Mites Recovered	Mite Mortality (Per cent)	Total No. Mites Recovered	Mite Mortality (Per cent)
1	681	15	505	8
2	1519	10	953	9
3	292	20	311	9
4	107	24	159	11
5	25	56	28	53
6	3	33	20	10
7	1	100	8	62
8	12	17	2	100
9	2	50	5	80
10	2	100	3	33
11	0	—	0	—
12	0	—	5	80
13	0	—	7	86
14	359*	44	1844*	24
Total	3003	17	3850	17

*Recovered after rats sacrificed

Table 3. Effects of 0.36% Dimethoate–treated Bait on Trombiculid Mites Infecting *Rattus rattus*.

Day	TEST ANIMALS (10)		CONTROL ANIMALS (10)	
	Total No. Mites Recovered	Mite Mortality (Per cent)	Total No. Mites Recovered	Mite Mortality (Per cent)
1	2300	10.5	632	0.2
2	162	86	846	0.3
3	29	93	94	9.5
4	6	100	71	0.6
5	1	100	20	5
6	1	100	7	14
7	0	—	27	7
8	0	—	13	7
9	0	—	0	—
10	273*	91	1153*	2
Total	2772	24	2863	3

*Recovered after rats sacrificed

Studies on the Growth, Development, and Reproduction of Gibbons in Captivity

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Associate Investigators: David E. Davidson, Jr., LTC, VC
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Yawalugsana Suttimool

OBJECTIVE: To collect information on the growth, development and reproduction of gibbons in captivity, and to collect normal biological data which may be useful in biomedical research.

BACKGROUND: A colony of gibbons (*Hylobates lar*) is maintained at SMRL for use in essential medical research projects of the laboratory. An active breeding program has been conducted for the past several years, and 28 young have been born in the colony. Physiolcgical and hematological observations are made on a regularly scheduled basis.

PROGRESS: Vaginal swabs were taken daily from nine adult gibbons and the time of menstruation was recorded. Fifty-two menstruations were observed. The duration of menstruation was 1–4 days, with 58 percent of these lasting only one day. The interval between menses was 17–120 days with the most common interval being 17–25 days. This information is similar to that recorded in previous annual reports. Information concerning copulation behavior and fetal development is being collected and will be reported when sufficient data are collected to allow interpretation. Since the last reporting period five gibbons were born in the colony. Birth data are listed in Table 1.

Table 1. Newborn Gibbons 1974–1975

Baby Number	Date of Birth	Parents	
		Female	Male
Pc 24	13 Sep 74	B–7	P–16
Pc 25	30 Oct 74	B–6	B–12
Pc 26	6 Jan 75	B–4	B–8
Pc 27	20 Feb 75	B–11	B–64
Pc 28	17 Mar 75	B–59	B–12

Colony-born gibbons have been examined monthly from birth to adulthood, and the sequence of tooth eruption has been recorded. Table 2 summarizes these observations to date.

Table 2. Tooth Eruption in Colony-born Gibbons--Mean Months ± 1 S.D.

Tooth	Deciduol	Permanent
1st incisor	1.0 (2)*	14.6 ± 1.5 (6)
2nd incisor	1.5 (2)	20.4 ± 2.2 (5)
Canine	3.4 ± 1.3 (7)	48.0 (1)
1st premolar	3.4 ± 0.5 (9)	31.0 (1)
2nd premolar	5.9 ± 1.3 (10)	41.0 (1)
1st molar	15.1 ± 1.6 (8)	41.0 (1)
2nd molar	18.0 (2)	41.0 (1)

*Numbers in parentheses represent total number of determinations

Blood Samples have been collected from all gibbons within the SMRL colony quarterly as a screening procedure for early detection of granulocytic leukemia and other diseases. This procedure has resulted in a large accumulation of hematologic data which have been compiled and are presented in Tables 3 and 4. Although many of the older gibbons in the colony were inoculated with various experimental agents during the late 1960's, the values presented represent samples taken from animals that were free of clinical evidence of experimental or spontaneous disease. The immature gibbons have not been used experimentally.

SUMMARY: Five gibbons were born in the breeding colony of eight mating pairs during the year. Observations on normal growth and development of colony born gibbons are presented and normal hematologic values in young and in mature gibbons are reported. These studies are continuing.

Table 3. Summary of Hematologic Findings in Mature Captive Gibbons – Mean Values ± 1 S.D.

Parameter		Non-splenectomized		Splenectomized	
		Male	Female	Male	Female
RBC × 10^6		7.70 ± 1.36 (385)*	8.02 ± 1.4 (260)	7.60 ± 1.29 (368)	7.12 ± 1.28 (384)
WBC × 10^3		11.32 ± 4.16 (489)	11.35 ± 5.76 (477)	12.46 ± 4.05 (426)	13.95 ± 4.92 (443)
PCV (%)		48.92 ± 5.93 (496)	48.13 ± 5.72 (339)	48.65 ± 4.93 (388)	46.77 ± 4.90 (507)
Hb (gm/100 ml)		14.74 ± 1.97 (439)	14.17 ± 1.78 (354)	14.53 ± 1.82 (321)	14.23 ± 1.70 (358)
Differential in percentage	Lymphocytes	53.19 ± 12.28 (493)	48.01 ± 16.52 (428)	51.21 ± 23.01 (410)	57.70 ± 33.31 (437)
	Neutrophils	43.47 ± 17.56 (491)	47.03 ± 19.78 (457)	39.92 ± 17.58 (404)	38.20 ± 15.10 (434)
	Basophils	1.05 ± 1.06 (491)	1.20 ± 1.18 (457)	1.18 ± 0.99 (404)	1.34 ± 1.33 (434)
	Eosinophils	2.29 ± 2.38 (491)	3.05 ± 3.06 (457)	2.48 ± 2.38 (404)	2.21 ± 2.17 (434)
	Monocytes	3.61 ± 2.29 (491)	4.00 ± 2.83 (457)	3.65 ± 2.60 (404)	3.41 ± 2.35 (434)
	Bands	1.18 ± 1.07 (491)	1.15 ± 0.70 (457)	1.21 ± 0.68 (404)	1.14 ± 0.60 (434)

* Numbers in parentheses represent total number of determinations.

*Table 4. Summary of Hematologic Findings in Immature Captive Gibbons (< 4 years old) – Mean ± 1 S.D.**

Parameter		Value
RBC x 10^6		7.18 ± 1.07 (49)**
WBC x 10^3		9.87 ± 1.58 (119)
PCV (%)		44.75 ± 4.48 (124)
Hb (gm/100 ml)		14.20 ± 1.57 (43)
Differential in Percentage	Lymphocytes	64.33 ± 13.39 (105)
	Neutrophils	31.19 ± 12.65 (105)
	Basophils	1.02 ± 0.78 (105)
	Eosinophils	2.44 ± 3.14 (105)
	Moncoytes	3.41 ± 2.13 (105)
	Bands	0.60 ± 0.32 (105)

* Values from both sexes included.

** Numbers in parentheses represent total examinations performed.

Laboratory Animal Disease in Thailand: Its Occurrence and Importance to Comparative Medicine

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OBJECTIVE: The objective of this study is to detect and investigate spontaneous diseases of laboratory animals. This information will aid in defining and improving the health of laboratory animals maintained in Thailand, and in developing animal models for the study of human diseases.

DESCRIPTION: In order to accomplish the objective, a program of continuous surveillance of the health status of the animal colony has been developed. Four areas are emphasized in this program: 1. the disease screening program conducted in the laboratory animal breeding colony, 2. the recurring clinical and laboratory examination of animals housed in the colony including those procedures performed during the quarantine of newly purchased animals, 3. the post mortem examination of animals that die in the colony, and 4. the development of standards for operation and quality control. When indicated by the findings, experimental studies are initiated to explore in detail the problems that occur.

PROGRESS: The prevalence of spontaneous infectious diseases in the rodent breeding colony remained at a low level during the year. This observation is consistent with findings during the previous reporting period. Annual production of mice, rats, hamsters and guinea pigs was reduced slightly due to a decrease in demand.

Disease screening was conducted quarterly utilizing retired breeders from the mouse, hamster and guinea pig production units. Results of histopathologic findings are summarized in Table 1. All lesions observed were mild and focal in nature and are not uncommon in old animals maintained under conventional conditions. Lesions observed were considered degenerative in nature, except the pulmonary lesions in mice which were consistent with chronic murine pneumonia, and the nematodes in the intestinal tract of mice which were identified as pinworms (*Aspicularis sp.*).

Table 1. Frequency of Histopathologic Findings in the Rodent Breeding Colony—1974.

Species	Number Examined	Pulmonary No. (%)	Gastro—Intestinal No. (%)	Genito—Urinary No. (%)	Hepatic No. (%)
Mouse	50	14 (28)*	5 (10)*	12 (24)	11 (22)
Hamster	30	0	2 (6)	2 (6)	4 (13)
Guinea Pig	35	14 (40)	0	3 (8.5)	6 (17)

* Nematodiasis

Bacteriologic examinations identified organisms similar in type and prevalence to those published in previous annual reports. Virologic screening studies were performed using sera from 50 retired breeder

mice. The hemagglutination–inhibition test (HI) was utilized for detecting antibodies to GD VII, Sendai, Reovirus 3, Minute Virus of Mice, Pneumonia Virus of Mice, K, and Polyoma viruses. Hepatitis, Lymphocytic Choriomeningitis, and Mouse Adenovirus antibodies were detected utilizing the Complement–fixation test (CF). Results of the HI tests are shown in Table 2. Results of the CF tests were not available at the time of publication.

During the period 1 April 1974 through 31 March 1975, 340 rhesus monkeys were imported directly from India. Twelve (3.5%) monkeys died during the quarantine period, primarily from gastrointestinal and respiratory diseases (see Table 3). Intestinal parasitism and measles were prevalent in newly arrived monkeys. One monkey that was sacrificed in February, 1975 after exhibiting clinical signs of a CNS disturbance, had a lobar pneumonia; a *Pneumococcus sp.* was isolated from the lungs.

Three gibbons in the SMRL colony died during the year. One adult male (S–58) died of pneumonia due to migrating *Strongyloides sp.* larvae. Two immature colony–born gibbons (PC 22, PC 23) died this year. Gibbon PC 22 died of a baterial pneumonia; no lesions were seen at necropsy examination which would account for the death of PC 23.

Table 2. Prevalence of Virus HI Antibody in 50 Mice

Virus Antibody	Percent Positive
GD VII	69
Sendai	60
Reovirus 3	60
Minute Virus of Mice	57
Pneumonia Virus of Mice	0
K	0
Polyoma	0

Table 3. Rhesus Monkey Losses During Quarantine
April 1974–March 1975

Month	Number Received	Number Deaths	Intestinal Disease	Pulmonary Disease	Undetermined
Apr 74	85	5 (5.9%)	3	2	0
Jul 74	85	3 (3.5%)	2	0	1
Feb 75	170	4 (2.3%)	1	3	0
Total	340	12 (3.5%)	6	5	1

Between October and December 1974, ten colony rabbits developed a moderate to servere necrotic dermatitis of the limbs. The infection generally began on the paw and progressed proximally. Clinically, the disease was characterized by swelling, redness, pain and loss of hair over the affected areas. Later the skin became necrotic and sloughed. In some rabbits the entire limb was involved, while in others only the distal portion of the limb was affected. Six animals responded to 14 days of Kanamycin therapy (15 mg/kg/day), while four animals did not respond and had to be sacrificed. Histologically, the disease was characterized as a necrotic or pyogranulomatous dermatitis with bacterial colonies visible in the necrotic debris. Bacteriologic examination revealed coagulase positive *Staphylococcus aureus*. Affected tissues were collected at necropsy, ground in a sterile glass Tenbrook grinder and inoculated into the dermis and subcutaneous tissue of the hindpaw of three clinically normal rabbits. One rabbit developed a subcutaneous abscess 12 days after inoculation which persisted until the animal was sacrificed at four weeks. *S. aureus* was recovered in pure culture. The portal of entry has not been clearly established, but may have been through the nail bed following trauma produced during toenail clipping operations.

Mice in the breeding colony were found to be infested with mites in October 1974. Alopecia, pruritus and inflammation were the predominant clinical signs. The mite was identified as *Myobia musculi*. All rooms were contaminated and all ages were affected. The condition was brought under control by whole body immersion of mice in a 2% malathion solution. Pregnant animals were not dipped until after their young were weaned. Baby mice were not dipped until the time of weaning. All bedding and cages were autoclaved before and after use for six weeks. Mortality from the dipping process was less than 0.4%. No evidence of mite infestation has been observed during the last three months.

Vertebrate Reservoirs of Disease

Principal Investigator: Joe T. Marshall, Jr., Ph.D.

Associate Investigators: Amara Markvong[1]
Vandee Nongngork

OBJECTIVE: To provide prompt identification of specimens and to advise concerning the ecology of vertebrates involved in the transmission of human disease.

BACKGROUND: Our large vertebrate collection was moved to the Applied Scientific Research Corporation of Thailand, where it became the nucleus of the Thai National Reference Collections under the curatorship of the late Mr. Kitti Thonglongya. A definitive collection of Asian rats and mice is still housed at SEATO Medical Research Laboratory.

Vertebrate studies starting with birds in the ecology of Japanese Encephalitis virus later shifted to mammals. Surprising discoveries in Thai mammals upset previous taxonomies, necessitating revisions throughout the entire Asian range of some genera. (Travel outside of Thailand was at personal expense).

DESCRIPTION: All possible evidence for species-limits has been assembled including scientific study-skins and skulls, tape recordings of vocalizations, observations of behavior and ecology in the natural state, host-specific ectoparasites, karyograms, and breeding experiments. Native mouse colonies have been distributed to laboratories at Yale, Roswell Park Memorial Institute, NIH, Houston, Woods Hole, Bonn, Cambridge, Lausanne, and New Zealand. At the moment we have requests for shipment of mice to Buffalo, NIH, Houston, and Hannover. The above laboratories are studying viruses, cancer, cytogentics, chromosome banding patterns, and satellite DNA. Our colony of the Asian house mouse, *Mus musculus castaneus*, has been especially valuable for cytologic and cancer research because of its wealth of novel alleles unknown in the laboratory house mouse.

PROGRESS: *Identification of Infected Animals.* Some identifications for various studies are listed in Table 1.

Taxonomic Research: Table 2 is an addition to the taxonomic checklist previously reported (1). Determination of the number of species of gibbons (Table 3) grew out of interest in rearing these apes at SEATO Medical Research Laboratory, where vocal distinctions were first noticed, and where karyotypes of three species were prepared. With E. Marshall, tape recordings were made of every species (in the wild except for *Hylobates concolor* (Figure 1). We discovered previously unknown areas of *Hylobates agilis* in Southern Thailand and Central Kalimantan (Figure 2). We are the first to possess data permitting a definitive enumeration of the species (Table 3).

REFERENCE:

1. Marshall, J. T.: SEATO Medical Research Laboratory Annual Report, April 1974

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† University of Arizona, Tucson, Arizona.

Table 1. Identification of Infected Animals

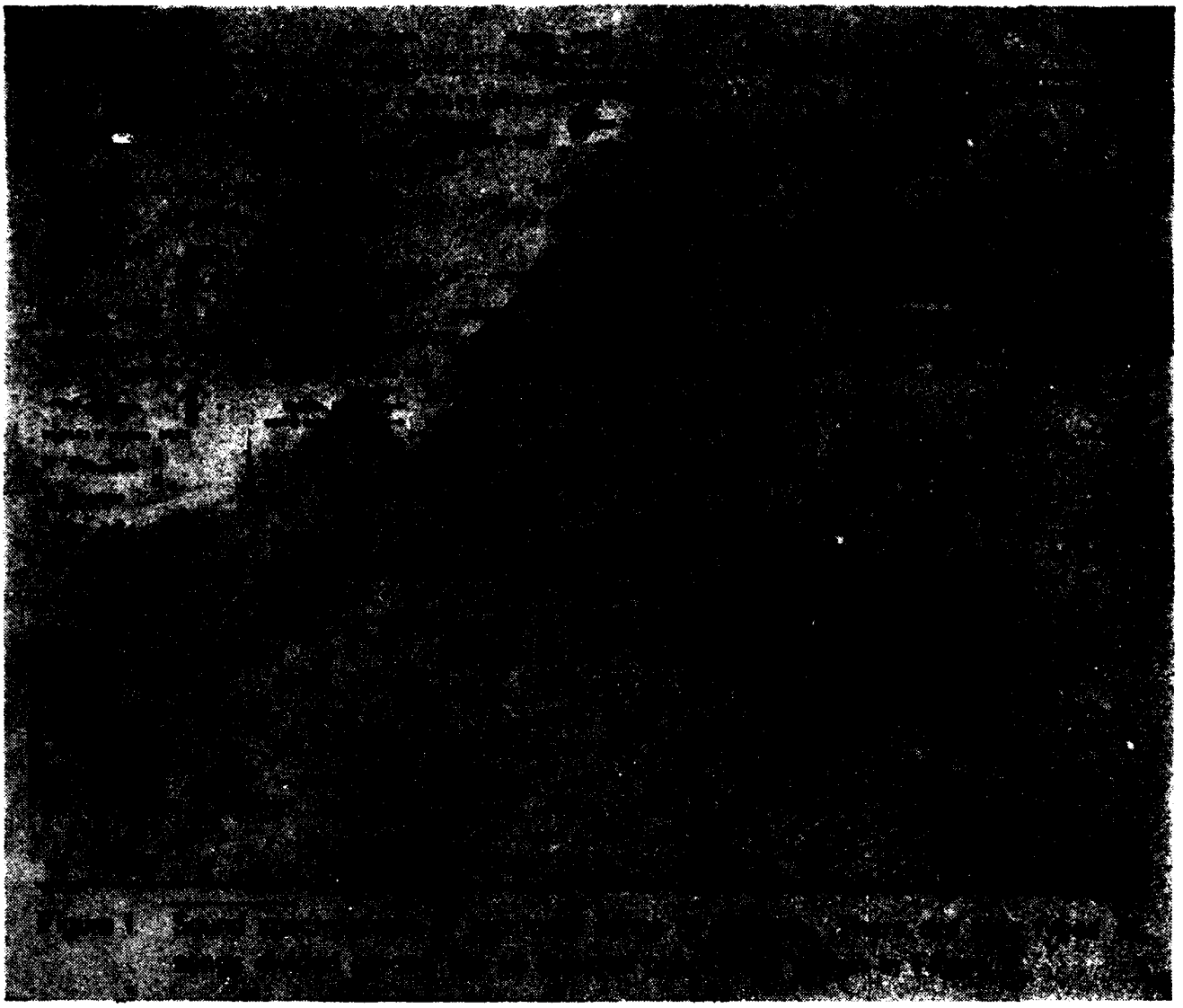
Study Project	Animals Identified	Remarks
Medico-ecology and Economic study, Nepal	*Bandicota bengalensis*	Large mounds in fields, burrows to 60 feet long, also in houses; first records for Kathmandu Valley high altitude villages; also fields at Kathmandu
	Mus musculus homourus	
	Mus musculus castanens	Houses at Kathmandu and Hetaura
	Rattus turkestanicus	High altitudes, in the house
	Rattus nitidus	Kathmandu, buildings
	Rattus brunneus	Buildings and fields, Kathmandu, Giant form of, or related to, *Rattus rattus*. Karyotype unlike *R. rattus* fide A. Gropp.
Rabies (positive, diagnosed by CPT Dill)	*Rattus rattus mindanensis*	Young male, no. 4-499; on 16 Sept 74 entered base bowling alley and bit sailor; Subic Bay, Philippines
	Rattus exulans	Host of fleas; indoors at Paktongchai and Bangkok
	Rattus rattus	Host of chiggers; forest at Sakaerat Station, fields at Paktongchai, houses and trees at Bangkok
Kaeng Khoi virus	*Rattus hinpoon* sp. nov.	Limestone cliffs, mouth of cave
	Rattus rattus	Cave mouth, also far inside
	Rattus neilli sp. nov. (Table 3)	Limestone cliffs, including mouth of cave
	Falco severus	A falcon which takes bats during their exit flight at dusk
	Taphozous theobaldi	A larger bat in the cave
	Tadarida plicata	The lesser, more numerous bat, and the one subject to fatal liver disease from the virus
Tick virus survey	Small carnivores, rodents	Hosts of ticks
Gnathostoma spinigerum	Mustelidae	Coordination of scientific names used by Russian authors
Rickettsiae; systemic poisons against ectoparasites	*Bandicota indica*	Hosts of ticks; fields at Paktongchai and Bangkok
	Bondicota savilei	Host of ticks; fields at Paktongchai
	Rattus surifer	Host of fleas; forest at Sakaerat Station
	Rattus norvegicus	Host of fleas; indoors at Paktongchai (Black phase) and Bangkok
	Rattus losea sakeratensis	No known ectoparasites; fields at Paktongchai

Table 2. Changes in Checklist of Thai Rats and Mice (1)

Former Name	New Name
Subgenus Leggad'lla	Subgenus Pyromys
Rajah Rats	Subgenus Maxomys
Niviventer Group	Subgenus Niviventer
Rattus niviventer	*Rattus confucianus*
Rattus fulvescens	*Rattus bukit*
Rattus fulvescens fulvescens	*Rattus bukit gracilis*
Rattus nielli is added, as number 37, in the Subgenus Leopoldamys.	
"*Rattus sakeratensis*"	*Rattus losea sakeratensis*

Table 3. Taxonomy of Gibbons (Lesser Apes), Genus *Hylobates*

Scientific Name	Common Name	Characteristic
A. Subgenus Symphalangus		50 chromosomes
1. *Hylobates syndactylus*	Siamang	
B. Subgenus Nomascus		52 chromosomes
2. *Hylobates concolor*	Hainan gibbon	
C. Subgenus Hylobates		44 chromosomes
I. Peripheral, isolated species		
3. *Hylobates moloch*	Javan gibbon	
4. *Hylobates hoolock*	Hoolock	
5. *Hylobates klossi*	Mentawai gibbon	
II. The Lar complex		
a. Superspecies of pileatus/muelleri		Bubbling great—call
6. *Hylobates pileatus*	Pileated gibbon	
7. *Hylobates muelleri*	Bornean gibbon	
b. Superspecies of agilis/lar		"Soaring" great—call
8. *Hylobates agilis*	Agile gibbon	
9. *Hylobates lar*	White-handed gibbon	



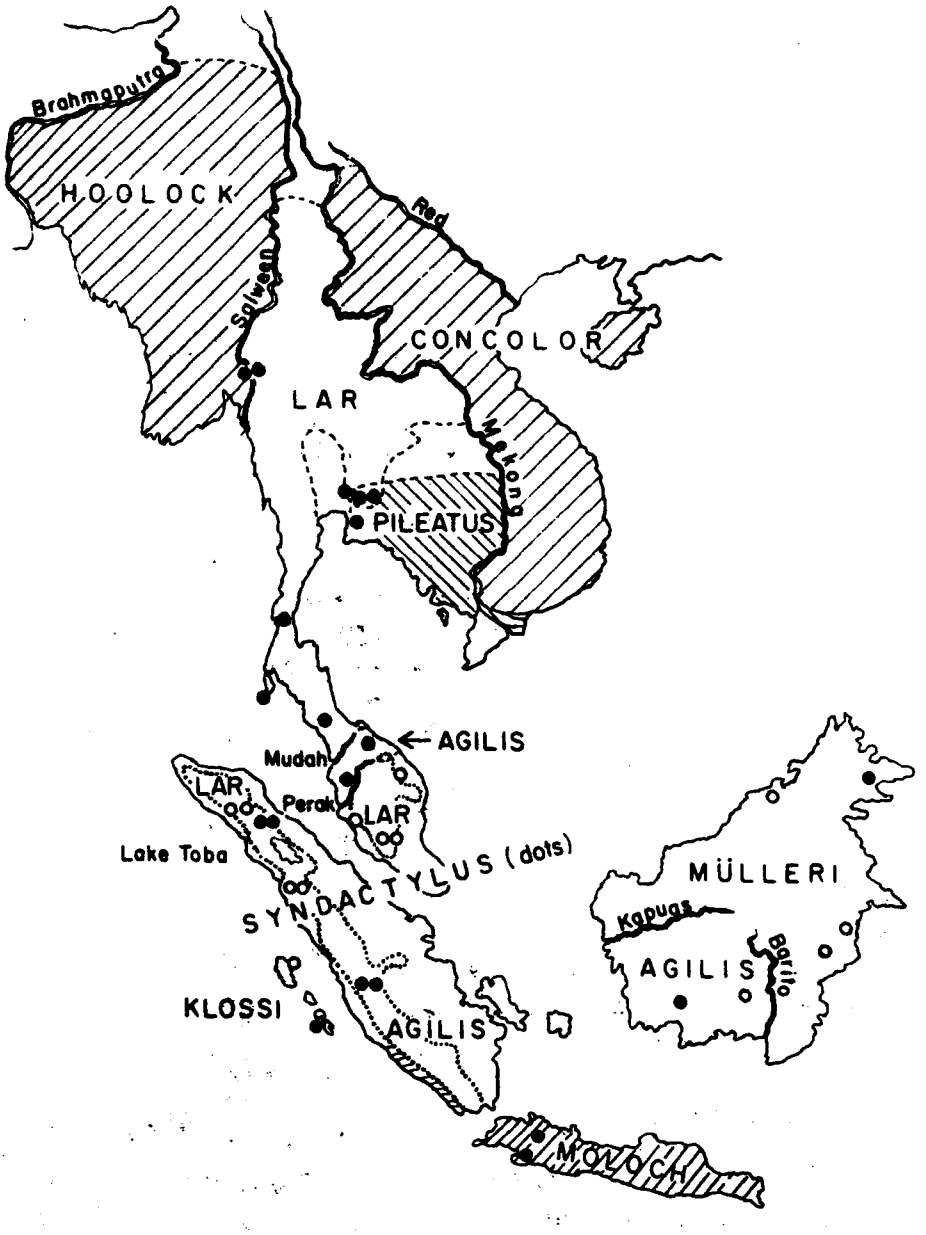


Figure 2. Distribution of species of gibbons. Solid circles indicate the places where the author tape-recorded wild gibbons; open circles are places whence recordings were supplied by colleagues.

Migratory Animal Pathological Survey

Principal Investigator: H. Elliott McClure, Ph.D.

OBJECTIVE: The objectives of the MAPS program as set out in the original proposal of 1963 was to learn the migration routes of birds of East Asia, the ectoparasites that were harboured on the birds and the haematozoa which infected birds as correlative data for use by epidemiologists interested in the dispersal and periodic occurrence of human infections, especially the arthropod borne viruses and rickettsias.

BACKGROUND: In October 1973 the Migratory Animal Pathological Survey activity was closed and all of the files and library moved from the Applied Scientific Research Corporation of Thailand (ASRCT) to the SEATO Medical Laboratory. A final report on the study of bird migration in Eastern Asia has been completed and published in book form (1). The first quarter of the year was devoted to proof reading and composition of the book which came off the press in June. More than 1,000 copies were mailed to ornithologists and libraries throughout the world and there has been a steady demand for copies ever since.

This report summarized the results from the banding of more than 1,200,000 birds of 1,218 species in ten countries of Eastern Asia; India, Thailand, Malaysia, Indonesia, Philippines, Taiwan, Hong Kong, Japan and Korea. There were more than seven thousand recoveries from these birds and their movements demonstrated that there were four major flyways across Asia, the studies being made in the East Asian and Indo—Asian flyways. The other two flyways are the Eastern European and Western European. Within these four flyways are numerous migration routes related to species and populations. Most species or populations remained in a given flyway without much overlap to adjacent ones. A given species with a wide distribution may have populations restricted to certain flyways and migration routes. Other information arising from these studies demonstrated that there was migration within the temperate zone and limited to it, migration within the tropics and limited to it, nomadism, altitudinal migration, and local dispersion as well.

Survival among the birds followed patterns already demonstrated in Europe and North America. Juvenile mortality is 60% or more until they gain experience. Following the first year survival rises until 75% or more live through each succeeding year. Data from Malaysia where there was a long term survival study indicated that many species were long lived, even tiny sunbirds which were still alive and active at 12 years.

PROGRESS: The work of MAPS was divided into three phases; bird migration, ectoparasites, and avian haematozoa. Summary of the ectoparasite studies appeared in book form in 1973(2). During the remainder of 1974 much time was devoted to the preparation of a report on the haemotozoa studies. These had involved the examination of more than 50,000 blood films from 1,147 species which had been completed before the project was closed at ASRCT. Summary, analysis, and revision are still underway and it is expected that the volume will be completed in 1975 or 1976 to make up the third report from this extensive study in Asia.

In the meantime recovery records continue to come in from hunters and bird students in Eastern Asia gradually increasing our knowledge of the survival of birds. Many species have now passed 10 years.

REFERENCES:

1. McClure, H.E.: Migration and Survival of the Birds of Asia, Bangkok: U.S. Army Component, SEATO Medical Research Laboratory, 1974, p 476.
2. McClure, H.E. and Ratanaworabhan, N.: Some Ectoparasites of the Birds of Asia, Bangkok: Applied Scientific Corporation of Thailand, 1973, p 219.

MALARIA STUDIES

The Suppression of *Plasmodium falciparum* and *Plasmodium vivax* Parasitemias by a Diformyldapsone – Pyrimethamine Combination

Principal Investigator: Eliot J. Pearlman, MAJ, MC

Associate Investigators: Withoon Thiemsaun
Ben F. Castaneda, SFC

OBJECTIVE: To study the effectiveness of the combination of diformyldapsone (DFD) 200 mg and pyrimethamine (Py) 12.5 mg in suppressing parasitemias in an area with known chloroquine resistant falciparum malaria.

BACKGROUND: The combination of dapsone (DDS) and pyrimethamine (Py) in the chemosuppression of chloroquine resistant falciparum malaria has been previously shown to be efficacious. The longer half life of the diformyl congener of dapsone should render this sulfone in combination with pyrimethamine a better chemosuppressive agent.

DESCRIPTION: Six hundred and fifty – nine semi – immune study subjects from three villages in Prachinburi Province, Northeast Thailand were assigned to one of five drug study groups. Subjects received a weekly medication, under a double blind design, of one of the following:

a. DFD 200 mg and Py 12.5 mg
b. DFD 400 mg
c. DDS 100 mg and Py 12.5 mg
d. Py 25 mg
e. Placebo

Each study subject was visited weekly; at which time the medication was given and swallowed under supervision; a capillary blood drawn for a thick – thin malaria smear; and a history of illness since the prior visit noted. Following the drug phase of the study four additional followup visits were made.

PROGRESS: Five hundred ninety – three study subjects (90%) completed the twenty – six week course of medication. Figure 1 shows that the weekly attack rates of new falciparum infections were lower during the medication phase of the trial in the sulfone groups (DFD – Py, DDS – Py, and DFD) compared to either the pyrimethamine or placebo group. It can be further seen that parasitemias were suppressed in the DFD – Py, DDS – Py, and DFD groups in the early phase of the study. An increased number of falciparum cases was seen in weeks 7 – 8 in both the DFD – Py and DFD groups, while a similar increase was seen in the DDS – Py group later (week 13).

Figure 2 shows the cumulative infection rates of individual study subjects in the course of the 26 week trial and the subsequent follow – ups. The data indicates a 4.6 fold reduction in the cumulative parasitemic rate for *P. falciparum* in the DFD – Py group and a 6.4 fold reduction in the DDS – Py group when compared to the placebo group.

The results of microscopy for falciparum parasitemias are given in Table 1.

Statistical evaluation comparing the various drug regimens was undertaken. Highly significant results were obtained which showed that DFD – Py, DDS – Py, and DFD alone were effective chemosuppressive agents against *P. falciparum* when compared with placebo alone ($0.0005 > p$). DFD – Py, as a combination, was more effective than its component parts: DFD ($0.0005 > p$) or Py ($0.0005 > p$) in suppressing a falciparum parasitemia. Significant differences, likewise, were seen when DDS – Py was compared with DFD alone ($0.0005 > p$), and Py alone ($0.0005 > p$); however, a direct comparison between DFD – Py and DDS – Py failed to reveal any significant difference in efficacy ($0.4875 > p > 0.475$). Table 2 summarizes these statistics.

Table 1. Results of Slide Microscopy* Drug Groups

Slide results	Pyrimethamine	DFD—Py	Placebo	DDS—Py	DFD
Negative slides	2139	2697	2015	2716	2528
Falciparum (P.f.t.) positive	433	27	449	28	58
Vivax (P.v.t.) positive	124	25	148	46	111
Mixed (P.f.t. + P.v.t.) positive	22	2	14	1	6
Total	2718	2751	2626	2791	2703

* Excluded are *P. falciparum* gametocytemia (P.f.g.) results and smears preceded the week before by absenteeism.

Table. 2 Calculated Student—t—values for Falciparum Parasitemias (P value)

	DFD	Py	Placebo	DDS—Py
DFD—Py	3.7470* (0.0005> p)	20.4216* (0.0005> p)	21.0752* (0.0005> p)	0.0553 (0.4875> p> 0.475)
DDS—Py	3.8181° (0.0005> p)	20.5830° (0.0005> p)	21.2391° (0.0005> p)	

* Values significant in favor of DFD—Py
° Values significant in favor of DDS—Py

While study subjects receiving the drugs: DDS—Py, DFD—Py or DFD alone were parasitemic less often (Table 3), no statistically significant difference ($X^2 = 2.1613$; $0.40 > p > 0.35$) in the density of falciparum parasites was seen (Table 4).

Table 3. *P. falciparum* Asexual Parasitemias Experienced by Study Subjects During Chemosuppression

Group	Number subjects	Number (prop.) infected	Total (average) number of episodes	Average duration (weeks) of episode
DDS—Py	123	12 (0.10)	17 (1.42)	1.42
DFD—Py	118	16 (0.14)	19 (1.19)	1.42
Diformyldapsone	117	26 (0.22)	42 (1.62)	1.43
Pyrimethamine	118	64 (0.54)	207 (3.23)	1.89
Palcebo	117	73 (0.62)	234 (3.21)	1.98

Table 4. Densities of *P. falciparum* Asexual Parasitemias Experienced by Study Subjects During Chemosuppression

Group	Number (proportion) parasitemias*	
	≤20	>20
DDS–Py	16 (0.94)	1 (0.06)
DFD–Py	16 (0.84)	3 (0.16)
Diformyldapsone	39 (0.93)	3 (0.07)
Pyrimethamine	180 (0.87)	27 (0.13)
Placebo	208 (0.89)	26 (0.11)

* In parasites per 100 white blood cells.

With cessation of chemosuppression the following new falciparum infections were seen in the five groups: thirteen in the DFD–Py group; eleven in the DFD group; fifteen in the DDS–Py group; one in the pyrimethamine group; and two in the placebo group. Of these post–treatment falciparum parasitemias, 24 of 42 occurred four months later. This would correspond to June, 1974, and the start of a new malaria transmission season.

A large number of vivax infections were seen in this study as shown in Table 5.

Table 5. *P. vivax* Parasitemia Experienced by Study Subjects During Chemosuppression

Group	Number of subjects	Number (prop.) infected
DDS–Py	123	17 (0.14)
DFD–Py	118	16 (0.14)
DFD	117	28 (0.24)
Py	117	52 (0.44)
Placebo	117	51 (0.44)

The Py and placebo groups each had a 44% cumulative vivax infection rate while the DFD–Py group had 14%, the DDS–Py group 14%, and the DFD group 24%. Statistical evaluation (Table 4) showed that the three sulfone groups suppressed vivax parasitemias better than pyrimethamine when compared with the control group. Py alone was totally ineffective. DFD–Py was not only more effective than its component parts: DFD ($0.0005 > p$) and Py ($0.0005 < p$), but DFD–Py was also more efficacious than DDS–Py ($0.01 > p > 0.005$) in the weekly chemosuppression of vivax malaria. DDS–Py likewise was more effective than DFD alone ($0.0005 > p$) and Py alone ($0.0005 > p$) in the weekly suppression of *P. vivax* parasitemias. Table 6 summarizes this information.

Table 6. Calculated Student–t–values for Vivax Parasitemias (p value)

	DFD	Py	Placebo	DDS–Py
DFD–Py	7.7092* (0.0005> p)	9.2758* (0.0005> p)	9.2758* (0.0005> p)	2.2793*
DDS–Py	5.7599° (0.0005> p)	7.4425° (0.0005> p)	8.5668° (0.0005> p)	–

* Values significant in favor of DFD–Py
° Values significant in favor of DDS–Py

Following completion of the chemosuppressive phase of the study there was an increase seen in new cases of vivax malaria in the DFD – Py group, 15; DDS – Py, 16; DFD, 11; Py, 4; and placebo, 5. The increase in the cumulative values for vivax infections are for the DFD–Py group from 0.14 to 0.29; for the DDS–Py group from 0.14 to 0.27; for the DFD group from 0.24 to 0.33; for the Py group 0.44 to 0.48 and for the placebo group fram 0.44 to 0.49.

SUMMARY: The combination DFD–Py given weekly was shown to be an effective chemosuppressive against both falciparum and vivax parasitemias, causing a four fold plus reduction in falciparum parasitemias, and an approximately three fold reduction in vivax parasitemias; however, this combination was not more efficacious than DDS–Py for the chemosuppression of falciparum malaria. DFD was only moderately effective, while there was no difference in chemosupperssion between pyrimethamine and placebo.

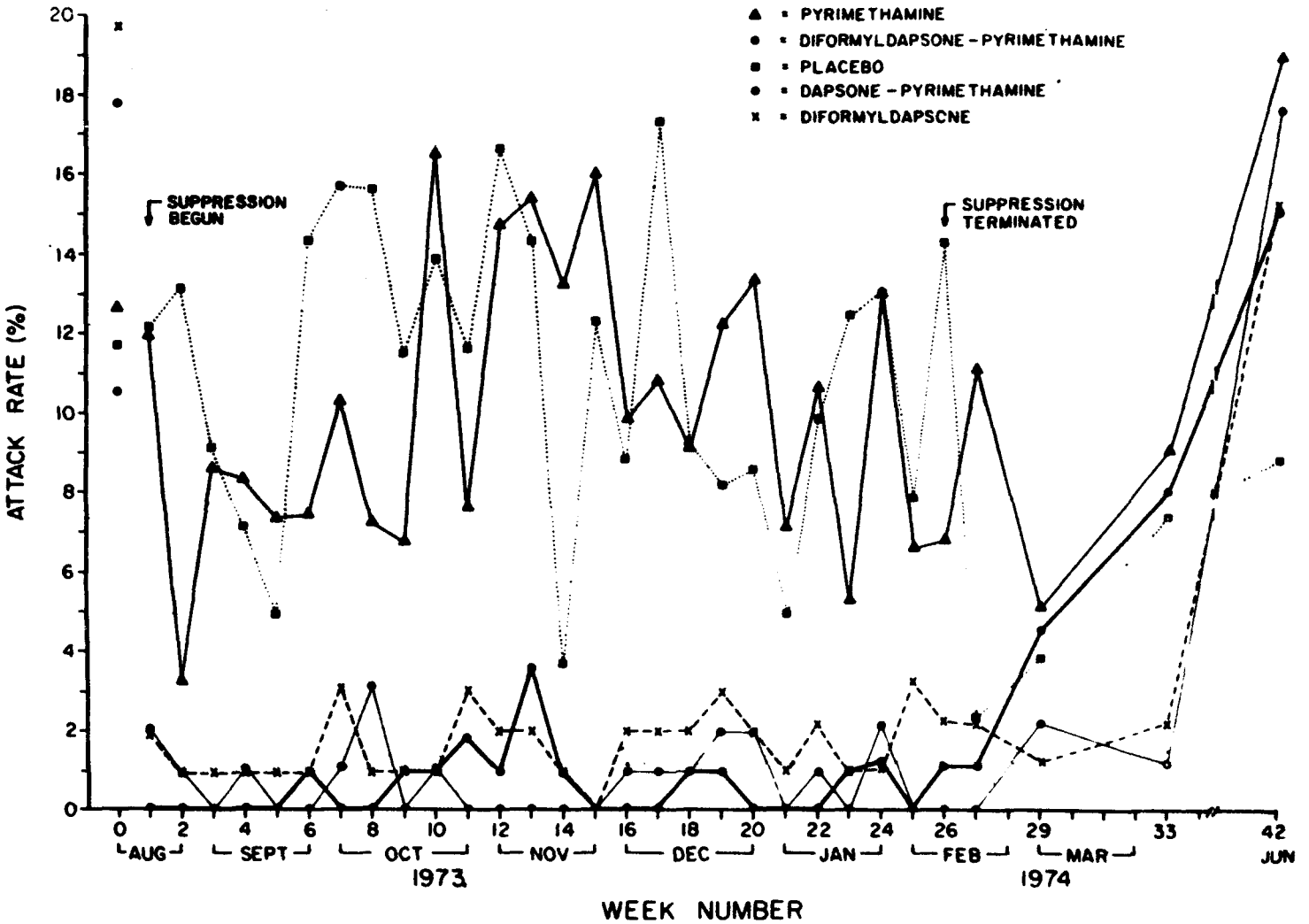


FIGURE 1. WEEKLY ATTACK RATE OF SUBJECTS INFECTED WITH P. FALCIPARUM BY STUDY GROUP

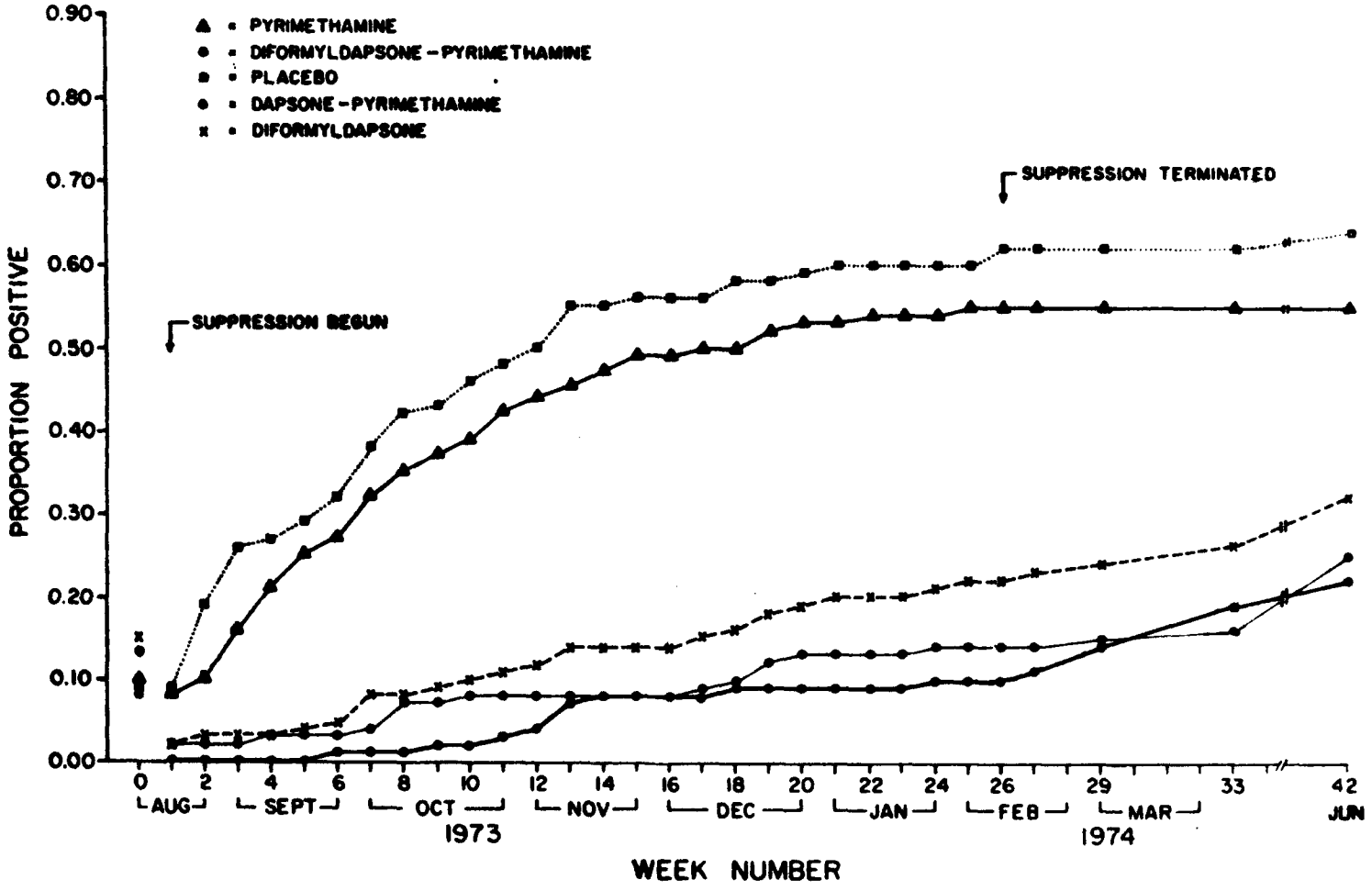


FIGURE 2 CUMULATIVE PROPORTION OF SUBJECTS INFECTED WITH P FALCIPARUM BY STUDY GROUP

The Suppression of *Plasmodium falciparum* and *Plasmodium vivax* Parasitemias by a Sulfadoxine – Pyrimethamine Combination

Principal Investigators: Eliot J. Pearlman, MAJ, MC
Richard M. Lampe, MAJ, MC

Associate Investigators: Withoon Thiemanun
Robert S. Kennedy, SFC

OBJECTIVE: To study the effectiveness of the combination of sulfadoxine (S) 500 mg and pyrimethamine (Py) 25 mg given in two dose regimens in suppressing parasitemias in an area with known chloroquine resistant falciparum malaria.

BACKGROUND: The combination of a sulfone or sulfonamide with pyrimethamine in the chemosuppression of chloroquine resistant falciparum malaria has been previously shown to be efficacious. The longer half life of a long acting sulfonamide, such as sulfadoxine ($t_{1/2} = 150 - 200$ hrs), should render this, in combination with a matched (in terms of $t_{1/2}$) dihydrofolic acid reductase, a better chemosuppressive agent.

DESCRIPTION: Seven hundred and fifty six semi-immune study subjects from four villages in Prachinburi Province, Northeast Thailand were assigned to one of five drug study groups. Subjects received, under a double blind design, one of the following medications:

a. Sulfadoxine 1000 mg – pyrimethamine 50 mg biweekly
b. Sulfadoxine 500 mg – pyrimethamine 25 mg biweekly
c. Diformyldapsone 200 mg – pyrimethamine 12.5 mg weekly
d. Diformyldapsone 400 mg – pyrimethamine 25 mg weekly
e. Placebo weekly

Each study subject was visited weekly, at which time the medication was given and swallowed under supervision, a capillary blood drawn for a thick-thin malaria smear, and a history of illness since the prior visit noted. For those subjects receiving a biweekly medication regimen, placebo tablets were given on the alternate weeks; thus study subjects received two tablets weekly.

PROGRESS: The twenty-six week course of medication phase of the study was concluded on 8 February 1975. Currently the study subjects are being monitored bimonthly for evidence of malaria transmission in the absence of chemosuppressive agents. At the termination of the medication phase, the average weekly study subject participation rate was approximately 88%. As the microscopy has not been completed, no data reduction is possible at this time.

SUMMARY: The combination of sulfadoxine and pyrimethamine has been given biweekly at two dosage levels for 26 weeks to semi-immune individuals in an area known to have chloroquine resistant falciparum malaria. The preliminary results of this study are not yet available.

Evaluation of the Sporonticidal Activity of Pyrimethamine–sulfadoxine (Fansidar) Against *P. falciparum* in Thailand

Principal Investigators: Edward B. Doberstyn, MAJ, MC
Douglas J. Gould, Ph.D.
Suphat Neoypatimanondh, M.D.
Stephen C. Hembree, CPT, MSC
Anthony P. Hall, COL, MC

OBJECTIVE: To determine the effect of single dose Fansidar therapy upon the subsequent development of oocysts and sporozoites of *P. falciparum* in vector mosquitoes and to correlate this information with plasma levels of pyrimethamine and sulfddoxine at the time of mosquito feeding. The activity of pyrimethamine alone will also be evaluated.

BACKGROUND: Fansidar, the fixed combination of pyrimethamine and sulfadoxine, has been shown to be very effective against chloroquine resistant and chloroquine sensitive strains of *P. falciparum* as well as *P. vivax* malaria in many parts of the world. It is currently recommended in Thailand as an alternative therapeutic regimen in the guidelines of the National Malaria Eradication Project. A number of studies (1, 2) have proven its effectiveness as a schizonticide, but it has not heretofore been adequately investigated as a gametocytocide and sporonticide. Chin, et al. (1) fed *Anopheles b. balabacensis* mosquitoes on patients following single–dose therapy; however, plasma levels of the constituent drugs were not determined at any time. In their series, 47% of mosquitoes fed on patients with gametocytemia showed development of the parasite up to the sporozoite stage, after feeding on days 7–9 and 13–14 following therapy.

In view of the already widespread use of Fansidar in the therapy of malaria in Southeast Asia, and its emergence as the drug of choice for chloroquine–resistant malaria in many parts of the world, the effect of this drug upon the infectivity of gametocytes needs to be conclusively determined. Epidemiologically, it is essential to be aware of the need for the additional use of a sporonticidal such as primaquine, in combination therapy.

DESCRIPTION: Patients presenting to the Malaria Eradication Service and district hospital outpatient clinics in Phrabuddhabat, Central Thailand, who are at least 15 years old and are found to have infections with *P. falciparum* are considered eligible for admission to the study.

Eligible patients are then randomly assigned to one of three groups: Group A: patients are treated with a single dose of Fansidar, two tablets (total 50 mg pyrimethamine, 1000 mg sulfadoxine). Group B: patients are treated with quinine, 10 grains every eight hours for six days. Group C: patients receive quinine as in Group B, plus pyrimethamine 50 mg daily for three days.

Fansidar, either alone or in combination with quinine, is the standard therapy for *P. falciparum* used in the outpatient clinic and on the wards in Prabuddhabat Hospital, and in the clinic operated by the National Malaria Eradication Project.

Medications are administered by the nursing staff, under the supervision of the study physicians. At the conclusion of the 21–day study period, patients from Group B and C are given two tablets of Fansidar. Recrudescences, if they occur, are re–treated with Quinine–Fansidar on an individualized basis.

Parasite counts are performed and blood is drawn for pyrimethamine and sulfadoxine levels before treatment is begun, daily in hospital and on days 5, 10, 15 and 20. Mosquito feeds are performed on days 0, 5, 10, 15 and 20 using colonized *A. b. balabacensis* from the SEATO Medical Research Laboratory

Phrabuddhabat Insectary. Patients are asked to return to the SEATO Medical Research Laboratory insectary for follow-up. If necessary, they are followed at home.

Ten percent of the mosquitoes fed on the patients are dissected ten days after feeding, and all mosquitoes are dissected on day 15, regardless of the results of the day 10 dissection.

Plasma pyrimethamine and sulfadoxine levels are performed at the SEATO Medical Research Laboratory Biochemistry Laboratory.

Numbers of mosquitoes developing oocysts and sporozoites will be evaluated as a function of plasma levels of drug at the time of feeding. Patients treated with quinine, which is known to have no effect on gametocytogeny or the development of mosquito forms, will provide the control population.

PROGRESS: Data collection is incomplete, and biochemical analyses are still pending, but it is becoming apparent that gametocytes from patients with detectable pyrimethamine and sulfa are infective to mosquitoes in some cases. To date, four of eight patients treated with the pyrimethamine-sulfadoxine combination developed gametocytes infective to the vector mosquitoes. All patients had detectable serum levels of pyrimethamine and sulfadoxine at the time of mosquito feeding.

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Hospital Survey of Malaria in Trad Province, Southeast Thailand

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OBJECTIVE: To conduct a comprehensive outpatient and inpatient survey of the clinical picture of falciparum and vivax malaria in a highly endemic area and to relate these findings to the results of therapy.

BACKGROUND: Certain parts of Trad Province are highly endemic for malaria and this disease accounts for 50% of all admissions to the Trad Hospital. SEATO studies were initially performed at the Hospital by Colwell and his team in 1970. The Rieckmann in-vitro test showed that the falciparum malaria was highly resistant to chloroquine. Extensive experience was also gained with the combination of quinine and tetracycline in the treatment of falciparum malaria. These studies terminated in 1971. In January 1973 an outpatient clinic was established at the Trad Hospital and maintained for an 18 month period.

DESCRIPTION: The SEATO outpatient clinic at Trad Hospital was open daily from 11 January 1973 to 21 July 1974. The patients were self-referred or referred by the Hospital staff. The subjects varied from healthy people requesting a blood checkup to patients in deep coma admitted to the ward and then examined. On each patient the following details were recorded: the SEATO OPD number, date, time, age, sex, name, asexual count, gametocyte count, history of malaria and the fact whether the patient had been born locally (local) or migrated from another part of Thailand (migrant). On patients with a positive malaria slide, a clinical history was taken, the temperature recorded and examination performed for splenomegaly. Selected patients positive for malaria were admitted to the ward and treated with one of the therapeutic regimens being evaluated. Further data was systematically collected. The data was entered on punch card transcripts and computer analysis will be performed. Complete data on an individual patient comprises the following: age, sex, parasite count in clinic, temperature in clinic, presence or absence of splenomegaly (in clinic or ward), migrant or local status, prior history, initial parasite count in hospital, maximum parasite count in hospital, parasite clearance time (in hours), initial temperature in hospital, temperature clearance time (in hours), lowest hematocrit, weight, serum bilirubin, serum creatinine, type of therapy and therapeutic result.

PROGRESS: During the study, 11,241 patients were screened for malaria in the clinic by means of a blood film. Falciparum malaria was diagnosed in 4,824 people and vivax malaria in 929 (Table 1). In 1973 the first peak for falciparum malaria (Figure 1) occurred in April (344 cases) and a second peak in November (359 cases). The incidence of vivax malaria showed an irregular fluctuation. The prevalence of malaria was much greater in 1974 and falciparum malaria peaked again in April (511 cases). Vivax malaria peaked in May 1974 (122 cases). About 950 patients with falciparum malaria were admitted to the ward and studied by the SEATO Lab.

Details of the therapeutic regimens used and results obtained are detailed elsewhere in this report. Computer analysis of all the data has not yet been completed. Preliminary analysis of the data has clearly shown that the average severity of the disease (as shown by the parasite count) was greater in the

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patients who had migrated from other parts of Thailand, in comparison with those born locally. Also the average level of parasitemia was lower in women and in old people. The largest group of patients with falciparum malaria had a parasite count between 10,000 and 100,000 per cu.mm. There was a positive correlation between the parasite count, the degree of anemia and the degree of jaundice. Clinically the average severity of the disease is worse during the seasonal peaks of incidence. It will be interesting to see whether the computer analysis supports this impression.

SUMMARY: Between January 1973 and July 1974, the SEATO laboratory maintained a malaria outpatient clinic in a highly endemic area of Southeast Thailand. The peak of malaria incidence was higher in 1974 than 1973. This confirms that malaria is still a serious problem in that area. Four thousand eight hundred patients were diagnosed in the clinic as having falciparum malaria and about 950 of these patients were admitted for therapeutic studies. A computer analysis of the outpatient and inpatient data is being prepared.

Table 1. SEATO Clinical Trad Hospital 1973—1974
Number of Patients with Falciparum and Vivax Malaria

Month	Patients Screened	Falciparum Malaria	Vivax Malaria
1973			
January*	167	75	11
February	318	102	14
March	618	278	44
April	743	344	38
May	642	252	43
June	585	221	46
July	537	225	37
August	603	208	51
September	436	103	31
October	292	71	13
November	590	359	41
December	483	268	25
Total	6014	2506	394
1974			
January	404	187	34
February	487	239	43
March	800	378	80
April	1071	511	112
May	1111	481	122
June	950	385	102
July**	404	137	42
1973—1974 Total—11241	11241	4824	929

* 11-31 January 1973
** 1-21 July 1974

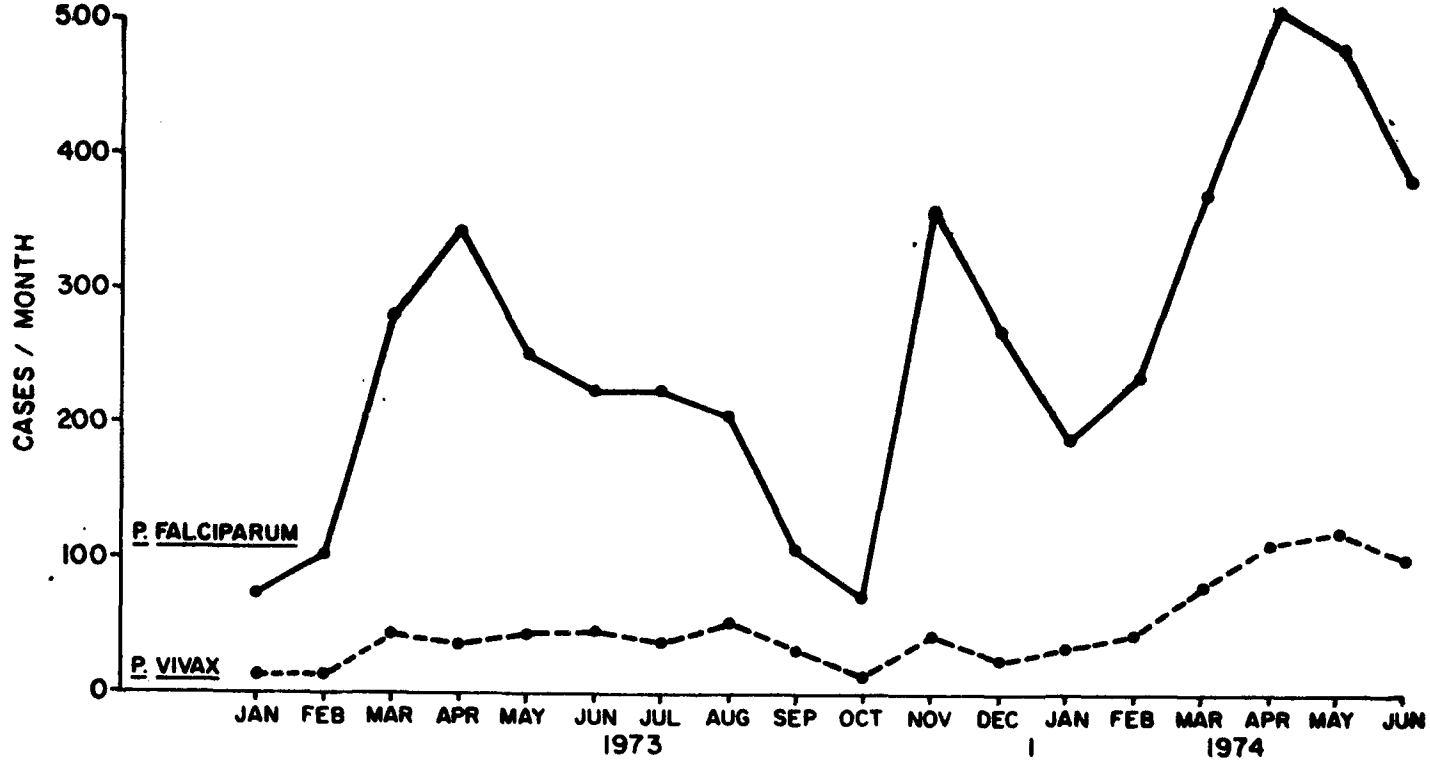

Figure 1. SEATO Malaria Clinic, Trad Hospital, 1973-1974. Number of patients with falciparum and vivax malaria.

Amodiaquine Resistant Falciparum Malaria in Thailand

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OBJECTIVE: To determine the comparative efficacy of amodiaquine and chloroquine against falciparum malaria in Thailand.

BACKGROUND: Interest in 4 – aminoquinolines other than chloroquine was reawakened by Schmidt as quoted by Rieckmann, (1) who found in owl monkeys that two chloroquine – resistant strains of *P. falciparum* were more susceptible to amodiaquine than to chloroquine. Rieckmann obtained similar findings both *in vitro* and *in vivo* although radical cures were not achieved in volunteers. Fitch demonstrated that owl monkey erythrocytes infected with chloroquine resistant *P. falciparum* had a deficiency of chloroquine – ^{14}C uptake, but not deficiency of amodiaquine – ^{14}C uptake (2). Therefore, we compared the therapeutic efficacy of the two drugs in a chloroquine – resistant endemic area. Preliminary results of this study have been tabulated (3).

DESCRIPTION: The study was performed at Trad Hospital in Southeast Thailand in 1973 and 1974. Details of the research methods have recently been given (3). The area is forested and malaria is endemic throughout the year. Chloroquine given either orally or parenterally (but not amodiaquine) is used frequently to treat patients with the clinical diagnosis of malaria. Chemoprophylaxis is not practiced in the community. All patients were fully informed on the nature of the drug trial and consent was granted voluntarily. They all had mild or moderate falciparum malaria and an asexual count greater than 1,000 per cu.mm. Alternate patients were assigned to chloroquine or amodiaquine. Chloroquine is 7 – chloro – 4 (4' – diethylamino – 1' – methyl butylamino) quinoline. The dosage form used was not enteric coated ("Nivaquine" tablets by May and Baker which contained 150 mg of chloroquine base). Amodiaquine is 7–chloro–4 (3, diethylamino methyl 1–4,–hydroxyanilino) quinoline, and the dosage form was a non–enteric coated tablet (Camoquine by Parke – Davis) which contained 200 mg of the base. A 1.5 g course of both drugs was administered during three days; 600 mg on the first day was followed by 300 mg six hours later and 300 mg on each of the succeeding two days.

Because only a minority of patients were cured by 1.5 g of amodiaquine given during three days, a 2.0 g course during four days was studied in an additional group of patients. Most patients received 400 mg (two tablets) initially followed by 400 mg six hours later on day 0, then 400 mg on the mornings of days 1, 2 and 3. All medications were administered by the study physicians.

Direct quantitative parasite counts (4) were performed before treatment, twice daily in hospital for at least three days and on days 14, 21 and 28 from the beginning of therapy. Daily hematocrits and leukocyte counts were done. Fifty milliliters of urine were obtained daily and frozen. The specimens were later analyzed for amino – quinoline content. Ten milliliters of urine (ph 8.3) were extracted with 25 ml of a 4:1 solvent of chloroform and isopropanol. Ten grams of anhydrous sodium sulfate were then added to the extract and the solutions filtered. The dried filtrates were dissolved in a few drops of methanol and then spotted on TLC plates precoated with silica gel. The plates were developed with a mixture of ethyl acetate, methanol and ammonium hydroxide (85:10:5) and then sprayed with acidified iodoplatinate. Control urines containing added chloroquine or amodiaquine were processed similarly and used as standards. The *Rf* for chloroquine was 0.85 and for amodiaquine was 0.96.

CHLOROQUINE 1.5 g. In hospital, chloroquine cleared parasitemia in only three of the 13 patients (Table 1) despite the mildness of many of the infections (average parasite count only 13,920). One of

these men developed a recrudescence in the follow-up period; the other two could not be traced. In many patients chloroquine had little or no effect on parasitemia—a potentially dangerous situation. Therefore, treatment with chloroquine was stopped after 13 patients and the final four patients received amodiaquine. The following case history illustrates the severity of chloroquine resistance in the patients studied.

Patient No. 7: This patient had a headache, myalgia and cough on admission. There was a fever of 39.5°C. Therapy with chloroquine 600 mg was given. On the next morning another 600 mg of chloroquine was administered. His parasitemia was 8,220 which increased to 18,270 in the evening. Another 300 mg of chloroquine was given at 1600 hours. The fever increased to 40.4°C and the patient became toxic and vomited. An intravenous infusion of quinine was given and the patient had greatly improved by the next morning. A ten dose course of quinine was administered, the fever and parasitemia cleared and the patient was asymptomatic when discharged; however, a recrudescence occurred on day 26.

Comment: Chloroquine 1.5 g was followed by an RIII response and a three day course of quinine was followed by an RI response.

AMODIAQUINE 1.5 g. The average total dose for the group was 30 mg/kg compared with 28 mg/kg for the chloroquine group. The standard 1.5 g course of amodiaquine was significantly more successful ($p < 0.01$) in clearing parasitemia (15 out of 17 patients) than chloroquine (Table 2). The mean parasite clearance time was 77 hours which, in relation to the mean parasite count of only 18,000 per cu.mm., can be considered prolonged. The mean fever clearance time was 47 hours which is unusually short for an antimalarial drug in our test system.

Four patients did not attend follow-up examinations and radical cure was achieved in 38 per cent (5/13) of the remainder. This is not a successful result because most of the patients had clinically mild disease.

AMODIAQUINE 2.0 g. The average total dose for the group was 42 mg/kg. Twenty-four patients with mild to moderate disease were treated. The parasitemia was cleared in 22 men (Table 3). The mean parasite clearance time was 77 hours. The mean fever clearance time was 36 hours which was much shorter than for any other regimen that we have tested, and probably indicates that amodiaquine does not cause a drug fever. The overall cure rate of 38 per cent (8/21) was the same as was obtained with the 1.5 g course of amodiaquine in the group of patients with a lower mean parasite count (Table 4). One patient had a clear-cut RIII response and another an RII response and the case histories are described below. In eleven patients the parasitemia cleared in hospital but a recrudescence occurred after discharge.

Patient No. 34: This patient was a 34 year old gem-miner who had migrated to the highly endemic area one month previously. He was probably non-immune. He had experienced headache and myalgia for nine days and was thirsty. His parasite count was 121,125 per cu.mm. His fever was 38.5°C on the day of admission and he received 600 mg amodiaquine followed by 200 mg four hours later. Despite another 400 mg amodiaquine at 0600 hours on day 1, his parasite count increased to 207,100 and his fever increased to 39.2°C with severe clinical toxicity. One dose of intravenous quinine therapy was given and then a single dose of pyrimethamine with sulfadoxine (Fansidar). His fever and parasitemia cleared but the patient did not attend follow-up.

Patient No. 37: This patient was a 38 year old farmer who had always lived in the endemic area. Therefore, using conventional terminology, he could be considered semi-immune. He had a headache for two days and had received one injection on the day before admission. His parasite count was 97,395 per cu.mm. and fever 39.4°C. On the day of admission 600 mg of amodiaquine was administered followed by 200 mg four hours later. Five hundred milliliters dextrose-saline was infused intravenously. On day 1 his headache persisted, as did a fever of 38-39°C; the parasitemia decreased to 4,845 per cu.mm. and another 400 mg of amodiaquine was administered. On day 2, despite two more doses of amodiaquine (to complete the 2.0 g course), the fever increased to 39.6°C and his parasite count rose to 36,480. A single dose of pyrimethamine with sulfadoxine was given and the temperature rapidly fell to 37.1°C. A radical cure was achieved.

The patient's falciparum malaria was resistant at the RII level to a 2.0 g course of amodiaquine given in five doses. The infection was then radically cured by a single dose of sulfadoxine 1.5 g with pyrimethamine 75 mg.

URINES: Urine specimens were obtained before treatment from 45 patients. Chromatography of these specimens showed spots corresponding to one or both of the 4-aminoquinolines in 39. The data do indicate that a high proportion of the patients had chloroquine therapy before admission.

All 45 patients had evidence of 4-aminoquinolines in post-treatment urine specimens. Chromatographic differentiation of the two drugs was not completely accurate.

TOXICITY: Symptoms (for example, nausea, abdominal discomfort and dizziness) were frequent during chloroquine therapy and were at least partially attributed to an unsatisfactory response to treatment.

Abdominal tightness, dizziness and other symptoms were fairly common on amodiaquine therapy (although not more so than with chloroquine) and in one patient the toxicity was alarming. Patient number 45 was aged 15 and weighed only 29 kg. He received a 2.0 g course during four days. Four hours after the last dose he complained of difficulty breathing and of palpitations. His pulse was normal but he appeared distressed and slightly cyanosed and had a prominent third heart sound. At this time his parasitemia was only 48 per cu.mm.; therefore, amodiaquine toxicity was the probable diagnosis. The patient improved within a few hours.

DISCUSSION: Falciparum malaria in Thailand responds poorly to chloroquine whether given as treatment (5,6) or for suppressive prophylaxis (7). The logical deduction is that chloroquine should not be used for falciparum malaria in Thailand. In practice, chloroquine is frequently prescribed both orally and parenterally, especially in remote areas, presumably because of low cost and easy supply. Whether this is desirable is a fundamental question. We do feel that due consideration should be given to banning the use of chloroquine in countries where falciparum malaria shows severe resistance as in Thailand (where the cure rate in our study was 0 per cent).

We found that vivax malaria comprises 14 per cent of all cases of malaria in Southeast Thailand (3). However, species identification is not usually available in the local laboratories. Thus, detection of vivax malaria is not normally achieved. Chloroquine, as a 1.5 g course over three days, is the appropriate treatment for the suppression of a clinical attack of vivax malaria. But without microscopic diagnosis, the patients are unlikely to receive a full course of chloroquine. In hospital the patients with vivax malaria receive the same therapy (which does not normally include chloroquine) as those with falciparum malaria. Therefore, if chloroquine were no longer available, the patients with vivax malaria (a benign disease) would not be treated differently in hospital and the patients with falciparum malaria (a serious disease), would not be receiving this ineffective drug before admission to hospital.

Another fact that has received insufficient attention is that both parenteral chloroquine (8,9) and oral chloroquine (10, 11) can be fatal. Cardiac arrest and convulsions are two of the most serious side-effects. High prolonged dosage can cause blindness and the indications for chloroquine therapy have been drastically reduced to malarias other than chloroquine resistant *P.falciparum* and extraintestinal amebiasis (12). Children are especially sensitive to the 4-aminoquinoline compounds. Amodiaquine can cause agranulocytosis (13) as well as toxicity similar to chloroquine. Fortunately in the treatment of malaria, 4-aminoquinolines by the oral route are relatively safe, especially in adults.

We found (Table 4) that amodiaquine (38 per cent cure rate) was more effective (Chi square = 4.16, $p < 0.05$) than chloroquine (0 per cent) in the treatment of chloroquine-resistant falciparum malaria. The predominant response was RI rather than RII. However, the cure rate of 38 per cent with multi-dose amodiaquine is not very impressive when compared with the 85 per cent cure rate we obtained with a single dose of pyrimethamine with sulfadoxine (14). The cure rates are significantly different (Chi square

= 14.8, $P < 0.01$). In the treatment of falciparum malaria in Thailand, quinine (3) or pyrimethamine with sulfadoxine (14, 15) are more effective than amodiaquine. Therefore, there is no clear-cut indication for amodiaquine in the treatment of falciparum malaria in Thailand. Amodiaquine might have an occasional role in patients who show hypersensitivity to quinine. But amodiaquine alone would not be satisfactory, since so many recrudescences occur. Following the initial course of amodiaquine, alternate therapy, such as a single dose of pyrimethamine with sulfadoxine, would be needed to prevent a recrudescence.

The fever clearance time was unusually short for amodiaquine compared with other antimalarial drugs which suggests that amodiaquine does not cause a drug fever.

The difference in cure rates between amodiaquine and chloroquine may by partly explained by the fact that chloroquine is widely used in Thailand whereas amodiaquine is not used at all. The falciparum parasites are probably resistant to 4-aminoquinolines in general but have not acquired a specific resistance to amodiaquine. Amodiaquine resistant falciparum malaria has also been detected in the Philippines where amodiaquine is used (16).

SUMMARY: Amodiaquine cured 38 per cent (13/34) of patients with falciparum malaria in Southeast Thailand. Chloroquine cured 0 per cent (0/13). The cure rates with amodiaquine were the same whether a 1.5 g or 2.0 g course was used. Most patients were resistant to amodiaquine at the RI level and to chloroquine at the RII level. In hospital amodiaquine cleared parasitemia more frequently than did chloroquine. With the 2.0 g course of amodiaquine, the parasite clearance time was 77 hours; the fever clearance time of 36 hours was short and suggests that amodiaquine does not cause a drug fever.

Because of resistance, chloroquine should not be used for falciparum malaria in Thailand. Routine use of amodiaquine is not indicated because more effective drugs are available.

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Table 1. Falciparum Malaria in Thailand. Therapy with 1.5 g Chloroquine over Three Days

Patient Number	Asexual Count *P. falciparum* (per cu.mm.)	Parasite Clearance Time (hours)	Initial Fever (°C)	Fever** Clearance Time (hours)	Result***	Comment
1	49572	—	38.5	—	RII	
2	27391	—	37.2	—	RII	
3	21021	—	38.8	52	RII	
4	20637	—	40.2	—	RII	
5	18200	69	36.8	—	—	
6	13160	—	40.0	—	RIII	
7	12820*	—	39.5	—	RIII	
8	6552	—	39.0	51	RII	
9	3240	—	37.7	—	RII	
10	2710	41	39.0	63	RI	
11	2340	—	37.5	—	RII	
12	1820	68	37.7	—	—	
13	1500	—	38.8	74	RII	
Mean	13920	N/A	38.5	N/A		Cure Rate=0% (0/11)

* Median count

** Fever clearance time not computed if initial fever 38.0°C

*** If no symbol, final result could not be determined. RIII, no marked reduction of asexual parasitemia; RII, marked reduction of asexual parasitemia, but no clearance; RI, clearance of asexual parasitemia, followed by recrudescence; S, clearance of asexual parasitemia without recrudescence (radical cure). World Health Organization (1967) Tech. Rep. Ser., No. 375, p.42.

Table 2. Falciparum Malaria in Thailand. Therapy with 1.5 g. Amodiaquine over Three Days

Patient Number	Asexual Count *P. falciparum* (Per cu.mm.)	Fever Clearance Time (hours)	Initial Fever (°C)	Fever Clearance Time (hours)	Result	Comment
14	54000	70	40.3	86	S	
15	35900	—	37.9	—	RII	
16	34830	60	39.5	46	S	
17	21060	75	38.0	43	—	
18	20000	69	38.0	56	RI	*P. vivax* Day 45
19	18428	70	37.3	—	RI	
20	18425	—	38.6	55	RII	
21	18200	100	38.2	20	S	
22	14256*	93	39.5	19	—	
23	13190	117	39.0	79	RI	
24	12376	76	37.7	—	RI	
25	9100	48	37.6	—	S	*P. vivax* Day 44
26	9100	65	39.7	13	—	
27	8730	90	37.5	—	RI	
28	7917	47	37.5	—	S	
29	7735	105	39.4	55	RI	
30	4320	66	37.7	—	—	
Mean	18092	77	38.4	47		Cure Rate=38% (5/13 followed-up cases)

* Median count

Table 3. Falciparum Malaria in Thailand. Therapy with 2.0 g Amodiaquine over Four Days

Patient Number	Asexual Count *P. falciparum* (per cu.mm.)	Parasite Clearance Time (hours)	Initial Fever (°C)	Parasite Clearance Time (hours)	Result	Comment
31	168720	—	39.8	64	S	
32	152000	85	39.8	60	RI	
33	149670	—	37.9	—	RI	
34	121125	—	38.5	—	RIII	
35	102980	88	37.4	—	RI**	
36	102600	—	38.4	40	S	
37	97395	—	39.4	—	RII**	
38	91960	86	39.7	61	RI	
39	65930	59	39.2	22	—	
40	63175	—	40.3	56	RI	
41	51680	—	39.3	6	RI	
42	48070*	116	38.2	43	RI	
43	45410	—	39.3	20	S	
44	42180	—	39.1	45	RI	
45	21140	75	39.8	43	—	
46	21090	87	38.6	14	RI	
47	16562	115	39.4	—	RI	
48	9696	88	37.3	—	RI	
49	7392	94	37.7	—	S	
50	5265	43	37.2	—	S	
51	4050	63	39.6	14	—	
52	3680	41	37.7	—	S	
53	2916	67	40.0	42	S	
54	2700	41	38.9	32	S	
Mean	58,228	77	38.9	36	Radical Cure Rate=38% (8/21)	

* Median count

** Cured by single dose pyrimethamine with sulfadoxine

Table 4. Comparison of Cure Rates

Drug	Mean Parasite Count (per cu.mm.)	RIII	RII	RI	S	Total	Cure* Rate
Chloroquine 1.5 g	14000	2	8	1	0	11	0 %
Amodiaquine 1.5 g	18000	0	2	6	5	13	38 %
Amodiaquine 2.0 g	58000	1	1	11	8	21	38 %

* Difference between cure rates for amodiaquine and chloroquine is significant ($\chi_2 = 4.23$, $p < 0.05$)

Falciparum Malaria Semi-resistant to Clindamycin

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BACKGROUND: The antimalarial activity of a group of chlorinated lincomycin analogues was first demonstrated in mice infected with *P. berghei* (6, 9) and in monkeys infected with *P. cynomolgi* (9, 11). Schizontocidal as well as causal prophylactic and radical curative activity was observed. Chloroquine-resistant *P. falciparum* infections in owl monkeys were also cured by these compounds (10). Both in animals and in man infected with malaria, clindamycin acted slowly; however, three day courses of quinine and clindamycin given in combination or sequentially proved effective against chloroquine-resistant falciparum malaria in volunteers (7). We tested clindamycin alone and in combination with quinine in Thais naturally infected with chloroquine-resistant falciparum malaria.

DESCRIPTION: The study was performed at the Trad Provincial Hospital in Southeast Thailand. Details of the research methods have been described (4). We operated a daily malaria clinic at the hospital and suitable outpatients volunteered for the inpatient studies. Informed consent was obtained in all cases. Male patients with an asexual parasite count over 1,000 per cu.mm. were included. To avoid the problem of immunity, patients with clinically mild infections were rarely studied.

Quantitative parasite counts were made at least twice daily in hospital on blood specimens obtained by finger-prick taken at 0700 and 1400 hours and at follow-up examinations on days 14, 21 and 28. Determination of the hematocrit (packed cell volume) and leukocyte count was made on admission and whenever clinically indicated. Sera were collected on admission and the concentrations of bilirubin and creatinine determined.

Throughout the study, the drugs were administered by one of the study physicians during medication-ward rounds usually made at 0600, 1400 and 2100 hours. The patients were observed by the physician as they swallowed the drug with water, then examined and kept under observation for a few minutes. The clindamycin was dispensed as 150 mg capsules (Cleocin, Upjohn) and the usual dose was 450 mg every 8 hours for three days (total dose 4050 mg). The quinine was administered as sugar-coated tablets of quinine sulfate, USP each containing 270 mg base. The usual dose was 540 mg every 8 hours for three days (total dose, 4860 mg).

Follow-up examinations on days 14, 21 and 28 were made either in the clinic or at home.

In the evaluation of the final therapeutic result in each patient, the WHO (14) classification was used (Table 1). A radical cure was diagnosed if the parasitemia was cleared and had not reappeared before day 29. Parasite clearance times were calculated in hours. Fever clearance times were computed in hours if the initial fever was at least 38.0°C. Clearance was diagnosed when the temperature decreased to 37.2°C or less and remained at this level for at least one more reading. If there was still fever or parasitemia on discharge at least 100 hours after admission, the elapsed time was arbitrarily counted as the clearance time.

CLINDAMYCIN: Eleven patients were treated with 450 mg every 8 hours for three days. One patient (Case No. 4) was a 12 year old boy weighing 28 kg. He received 300 mg every 8 hours. In five patients not responding to clindamycin, the drug was stopped and more effective therapy given.

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The initial clinical response was fairly rapid in some of the patients (Table 1) but the mean parasite clearance time was prolonged (88 hours) as was the fever clearance time (68 hours).

In five patients the parasitemia was cleared and did not reappear on follow-up examination on days 14, 21 and 28. These patients were adjudged to be radically cured. The average initial parasite count in these five patients (33269 per cu.mm.) was less than that (73511 per cu.mm.) in the five patients who were not cured (the difference was not statistically significant). In two other patients (Cases 2 and 6) an initial clinical response occurred but follow-up was not achieved. Thus the initial clinical response was satisfactory in seven of the 12 patients.

In five patients the initial infection was not controlled by clindamycin and because of a worsening clinical and parasitemic situation, alternate therapy had to be given. Two of these patients were cured by sequential therapy with quinine and Fansidar (pyrimethamine and sulfadoxine). Two other patients were cured by a single dose of Fansidar given alone. The case-history in one of these patients is described below. A clinical response to Fansidar occurred in the fifth patient but follow-up was not obtained. Thus the overall cure rate for clindamycin was 50% (5/10).

No clear-cut toxicity due to clindamycin occurred in these patients; however, Case No. 2 had persistent dizziness and weakness during therapy and tinnitus occurred for two days afterwards.

Case No. 12: The patient was a 43 year old farmer born locally. The main symptom was a headache for four days. He had received two intramuscular injections (content unknown) on the day before admission. He was in distress with a fever of 40.0°C, although his parasitemia was only 8265 per cu.mm. Clindamycin 450 mg was administered at 1600 and 2100 on day 0 and thereafter every eight hours. The fever abated briefly on the morning of day 1 but then returned to 40.0°C. The parasitemia decreased to 665 on day 1 but then rose to 9880 on day 2. On the evening of day 2, because of the increase in parasitemia, the high fever (40.0°C) and persistent severe symptoms, drug failure (RII type) was diagnosed. Seven doses of clindamycin had been given. The patient then received a single dose of Fansidar (sulfadoxine 1.5 g with pyrimethamine 75 mg). The pasasitemia then cleared in 48 hours and the fever in 60 hours. Blood films were negative on days 13, 21 and 30 and a radical cure was diagnosed.

QUININE WITH CLINDAMYCIN (FULL DOSAGE): Six patients were begun on treatment with quinine 540 mg base every 8 hours and clindamycin 450 mg every 8 hours given at the same time for three days (Table 2). The dosage of clindamycin was reduced (usually to 300 mg) in most patients because of intolerance.

In five of the six patients, the quinine-clindamycin combination appeared to cause toxicity. In Case No. 13; his symptoms worsened during therapy but improved when the clindamycin was stopped. In Case No. 15; despite a fall in parasite count from 55,419 to 40, he developed severe anorexia after the sixth dose of clindamycin. Cases No. 16, 17 and 18 had a similar clinical picture; they developed severe retching one to four hours after the second to fourth dose of quinine and clindamycin. They all improved after the clindamycin was stopped despite the continuation of the quinine at full dosage. After the course of quinine was finished and the patients had improved, clindamycin was resumed without causing any side effects when given alone.

Four patients were cured and two others did not complete follow-up. The mean parasite clearance time was prolonged (83 hours) and the mean fever clearance time was 67 hours.

QUININE WITH CLINDAMYCIN (HALF DOSAGE): Eight patients received combination therapy with half dose quinine (270 mg) and approximately half-dose clindamycin (150 mg) every 8 hours (Table 3). The mean parasite clearance time was prolonged (95 hours) and the mean fever clearance time was 68 hours. Complete follow-up was achieved in five patients of whom three were cured (60%). A clinical response occurred in two other patients but follow-up was not achieved. The eighth patient (Case No. 22)

developed toxicity after four doses of therapy and was then treated with quinine followed by Fansidar. Five of the eight patients developed unacceptable toxicity which consisted mainly of upper gastrointestinal symptoms. Details of three of these cases are given.

Case No. 19: This patient received four full doses of quinine (three intravenously) without toxicity. He then received quinine with clindamycin at half dosage. After two doses the patient developed nausea, tightness in the chest and severe retching which persisted for eight hours. The clindamycin was stopped. The symptoms improved although the quinine therapy was continued. After the nine dose course of quinine had been completed, the clindamycin was resumed until nine doses had been given. The patient had persistent weakness during this time. The parasitemia and fever cleared but follow-up until day 28 was not obtained.

Case No. 20: This patient received five full doses of quinine at eight hour intervals uneventfully. Then the semi-dose combination was given for two doses. The patient felt generally worse and a persistent fever developed. The clindamycin was stopped and also the quinine after two more doses. Sixteen hours later when he felt better the clindamycin was resumed for five doses during which time the patient felt listless. A radical cure was achieved.

Case No. 22: The patient was virtually asymptomatic on admission, having mild headache and backache only. After two doses of quinine 540 mg and clindamycin 300 mg at 1000 and 2100 hours on day 0, he developed nausea and vomiting, headache, dizziness and prostration. On day 1 he received quinine 270 mg and clindamycin 150 mg at 0600 and 1400. The patient remained very toxic with weakness and dizziness although the parasite count had fallen from 60,000 to 650 per cu.mm. The clindamycin was stopped but the quinine was continued and the dose increased to 540 mg; the patient improved and remained well. After 12 doses of quinine, a single dose of Fansidar was given. The patient was cured.

QUININE ALONE: Three patients were treated with a three day course of quinine alone. They developed recrudescences on days 14, 22 and 25, respectively (Table 4).

TETRACYCLINE ALONE: Four patients received tetracycline 250 mg every 6 hours for three days (Table 5). Two patients had an RIII response and one patient had an RII response. A slow clearance of parasitemia occurred in the fourth patient but follow-up was not obtained.

DISCUSSION: Falciparum malaria in Thailand is difficult to eradicate in many patients. In recent studies the cure rate with chloroquine (5) or with pyrimethamine (3) was 0%. This data indicates that clindamycin is partially effective against chloroquine-resistant falciparum malaria in patients with clinically moderate disease. In some men the clinical response to clindamycin was rapid but in others it was slow or ephemeral. Our cure rate of 50% with multi-dose clindamycin compares with the 85% cure rate we obtained with a single dose of pyrimethamine with sulfadoxine (3). The average parasite clearance time for clindamycin (88 hours) was significantly longer ($p < 0.05$) than that for pyrimethamine with sulfadoxine (71 hours). Wagner et al. (13) found that clindamycin has a short half-life of only 2.4 hours and consequently, frequent doses must be given which is a disadvantage in the treatment of malaria. On the other hand sulfadoxine has a half-life of about 200 hours as determined by Brooks et al. (1). In our test system clindamycin was obviously a more powerful antimalarial than tetracycline (Table 5).

Clindamycin alone was not toxic in our patients; however, several recent reports have shown that lincomycin or clindamycin (a chlorinated lincomycin analogue) can cause ulcerative colitis or even pseudomembranous colitis (2, 8, 12). The diarrhea usually begins after 4 to 9 days of therapy. Colitis was not detected in our patients. Clindamycin is probably the most potent antimalarial among the antibiotics. However, because of its partial efficacy and potential toxicity, clindamycin alone has a limited role as an antimalarial.

Quinine and clindamycin in combination at full or half dosage apparently potentiated toxicity in our patients. Retching and frank vomiting were frequently observed, although Miller et al. (7) did not encounter gastrointestinal intolerance. Other patients had less specific symptoms and did not look well.

When the clindamycin was stopped but the quinine continued, the patients improved. Likewise when the course of quinine had been completed, clindamycin alone did not cause serious side-effects. The therapeutic results with full dose quinine and clindamycin therapy were excellent (4/4 cures). Perhaps quinine and clindamycin potentiate both antimalarial efficacy and toxicity.

Sequential administration of quinine and clindamycin was not toxic and could be useful in patients who have relapsed following more conventional therapy (e.g. quinine followed by Fansidar).

By studying high count rather than low count cases, we produced a more severe test of antimalarial efficacy in any regimen studied i.e, the degree of "drug pressure" was increased. If patients with counts only over 50,000 per cu.mm. and uncomplicated disease are selected for drug trials, comparative studies can be completed using fewer subjects. We have adopted this system without risk to the patients.

SUMMARY: Clindamycin, a semi-synthetic antibiotic of the lincomycin family, at a dose of 450 mg every 8 hours for three days in adults, cured 50% (5/10) of patients moderately ill with chloroquine-resistant falciparum malaria. Combination therapy with full dose quinine and clindamycin for three days was curative in 100% (four patients) and with half-dosage in 60% (3/5). However both combinations caused upper gastrointestinal toxicity and appeared to potentiate both toxicity and possibly antimalarial efficacy. Colitis due to clindamycin was not observed. Sequential therapy was not toxic and could be useful in patients who have recrudesced following more conventional therapy.

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Table 1. Falciparum Malaria Therapy with Clindamycin Every 8 Hours for 3 Days

Patient Number	Asexual Count *P. falciparum* (per cu.mm.)	Parasite Clearance Time (Hours)	Initial Fever (°C)	Fever Clearance Time (Hours)	Result	Comment
1	162260	—**	39.8	—	RIII	Cured by Quinine and Fansidar
2	93670	99	39.9	55	—	
3	82080	87	40.4	63	S	
4	77900	—	37.9	—	RII	Cured by Quinine and Fansidar
5	72770	—	40.5	—	RII	
6	51300	—	40.2	54	—	
7	46360*	—	40.0	—	RII	Cured by Fansidar
8	33060	97	40.0	102+	S	
9	20140	88	40.7	63	S	
10	18144	92	39.9	76	S	
11	12920	67	40.0	66	S	
12	8265	—	40.0	—	RIII	Cured by Fansidar
MEAN	56572	88	40.0	68	Radical Cure Rate=50% (5/10)	

* Approximately the median count.

** If no symbol, final result could not be determined. RIII, no marked reduction of asexual parasitemia, but no clearance; RII, marked reduction of asexual parasitemia, but no clearance; RI, clearance of asexual parasitemia, followed by recrudescence; S, clearance of asexual parasitemia, without recrudescence (radical cure).

Table 2. Falciparum Malaria Therapy with Quinine and Clindamycin Both Given Every 8 Hours for 3 Days at Full Dosage

Patient Number	Asexual Count *P. falciparum* (per cu.mm.)	Parasite Clearance Time (Hours)	Initial Fever (°C)	Fever Clearance Time (Hours)	Result	Comment
13	146692	117	38.3	82	S	Drug Toxicity
14	101556	93	39.6	60	S	
15	55419	64	40.0	39	S	Anorexia
16	33943*	85	40.6	60	—	Vomiting
17	8645	69	40.0	69	—	Vomiting
18	5642	69	40.0	92	S	Vomiting
MEAN	58,650	83	39.8	67	Radical Cure Rate = 100% (4/4)	

* Approximately the median count

Table 3. Falciparum Malaria Therapy with Quinine and Clindamycin Both Given Every 8 Hours for 3 Days at Half Dosage

Patient Number	Asexual Count *P. falciparum* (per cu.mm.)	Parasite Clearance Time (Hours)	Initial fever (°C)	Fever Clearance Time (Hours)	Result	Comment
19	358,830	119	37.5	99	—	Vomiting
20	236,600	116	39.1	115+	S	Drug fever
21	214,760	115	38.5	42	RI	
22	61,880	—	38.0	—	—	Toxicity
23	54,432*	67	39.9	18	S	
24	13,312	92	38.9	32	—	Vomiting
25	4,914	96	40.2	112+	S	Abdominal pain
26	2,730	59	40.9	58	RI	
MEAN	118,432	95	39.1	68	Radical Cure Rate = 60% (3/5)	

* Approximately the median count

Table 4. Falciparum Malaria Therapy with Quinine Every 8 Hours for 3 Days

Patient Number	Asexual Count *P. falciparum* (per cu.mm.)	Parasite Clearance Time (Hours)	Initial Fever (°C)	Fever Clearance Time (Hours)	Result
27	87,804	84	37.9	—	RI
28	21,708	69	39.8	—	RI
29	18,270	68	40.5	84	RI
MEAN	42,594	74	39.4	—	

Table 5. Falciparum Malaria Therapeutic Results in Patients Treated with Clindamycin or Tetracycline*

Regimen	Duration Therapy	Average Parasitemia	RIII	RII	RI	S
Clindamycin	3 Days	53389	1	4	0	5
Tetracycline**	3 Days	20647	2	1	—	—

* Clinically clindamycin was more effective than tetracycline.

** One patient responded in hospital but follow—up was not achieved.

Single--Dose Therapy of Falciparum Malaria Using Pyrimethamine in Combination with Diformyldapsone or Sulfadoxine

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OBJECTIVE: To compare the efficacy of diformyldapsone and sulfadoxine used with pyrimethamine in the treatment of falciparum malaria.

BACKGROUND: Weekly administration of pyrimethamine and diformyldapsone (DFD) has proved to be an effective chemosuppressant against drug–resistant *P. falciparum* malaria in both induced infection in volunteers (1) and in field populations (2).

In a trial of DFD alone as therapy for falciparum malaria, Clyde, et al, found that single dose treatment was slowly effective in clearing 15 of 23 episodes of asexual parasitemia in volunteers; however, recrudescence occurred in all but one patient (3).

The combination of pyrimethamine with sulfadoxine (Fansidar) has been reported to be highly effective both for treatment (4) and prophylaxis (5) of drug–resistant falciparum malaria.

Pyrimethamine–DFD, like pyrimethamine–sulfadoxine, is a single–dose preparation, and the therapeutic potency of the two combinations was compared.

DESCRIPTION: The patients to be studied were selected from males presenting at the Trad Provincial Hospital in Southeast Thailand with an asexual parasite count of *P. falciparum* greater than 1000 per cu. mm. The patients also had to agree to be hospitalized and followed during the study period, and be willing to signify consent after being informed of the nature and potential hazards of the study. Since pyrimethamine with sulfadoxine is known to be therapeutically effective, 45 patients were selected who had clinically moderate disease. Their average age was 22.7 years and average weight 47.3 kg. Since the therapeutic potency of pyrimethamine with DFD was not known, most of the patients selected had clinically mild disease. Thirty three patients received pyrimethamine with DFD. Their average age was 23.6 years and average weight 51.5 kg.

Direct quantitative parasite counts (6) were performed on admission and thereafter twice daily in hospital and 14, 21 and 28 days after admission. Hematocrit, white cell count, and urinalysis were performed on admission, and whenever subsequently indicated. Patients were taken home by a member of the study team, and follow–up examinations were made either at the clinic or at the patients' homes. Other details of the study procedure are described elsewhere (7).

All medications were administered by one of the study physicians, and patients were seen on clinical rounds made three times daily. The drugs used were combination tablets of pyrimethamine 25 mg and sulfadoxine 500 mg (Fansidar, Hoffmann–LaRoche) or pyrimethamine 12.5 mg and diformyldapsone 200 mg (supplied by the Walter Reed Army Institute of Research). Both were supplied as uncoated white tablets. The dosage of pyrimethamine–sulfadoxine used in adults was pyrimethamine 75 mg and sulfadoxine 1500 mg (3 tablets). This is the maximum dose recommended by the manufacturer. Seven boys weighing between 20 and 36 kg received two tablets. One boy weighing 18 kg received one tablet. Two dosages of the pyrimethamine–DFD combination were tested: pyrimethamine 25 mg, DFD 400 mg (2 tablets) and pyrimethamine 50 mg, DFD 800 mg (4 tablets).

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Therapeutic responses were classified according to WHO criteria (8). Parasite clearance times and fever clearance times were determined for each patient. Fever was considered to be "cleared" if it remained at or below 37.2°C for at least 12 hours. Patients in whom asexual parasitemia was cleared by treatment and had not reappeared for 28 days following therapy were considered radically cured.

PROGRESS: PYRIMETHAMINE 75 MG, SULFADOXINE 1500 MG.

Forty–five patients were treated with the combination pyrimethamine–sulfadoxine (Table 1). Patients in this group had a high average parasitemia (60,000 per cu.mm.) and were moderately or severely ill. The mean parasite clearance time was 73 hours and the mean fever clearance time was 63 hours.

Thirty–nine of the forty–five men were followed for the 28 day observation period and radical cure was attained in 33 (85%). All patients with parasite counts below 30,000 per cu.mm. were cured. A typical successful response to pyrimethamine–sulfadoxine is described below.

Patient No. 5: This 18 year old farmer was admitted with a three day history of fever, insomnia, and backache. He gave a history of malaria one year previously. On physical examination, he was found to have a temperature of 39.2°C, but no other positive findings. The asexual parasite density of *P. falciparum* was 115,900 per cu.mm. on admission. He remained febrile for 51 hours following treatment and complained of continuing backache, but his parasitemia steadily decreased. There were rare ring forms 48 hours after therapy, but smears were negative thereafter. He remained well throughout the follow–up period.

Two patients exhibited recrudescence of asexual parasitemia and malaria symptoms before the end of the 28 days and were considered RI treatment failures. Six patients required intravenous quinine after the initiation of the pyrimethamine–sulfadoxine therapy because of rising parasitemia and worsening clinical state. However, to bring the infection under control, four patients required only one dose of quinine one received two doses and another five doses. Some of the patients may indeed have eventually responded to the single dose of Fansidar, but in the judgement of the attending physicians, withholding the faster acting drug of known effectiveness was not warranted. Four patients were diagnosed as RIII treatment failures. In two patients (Numbers 1 and 4), it was later considered that the quinine therapy may have been given prematurely, so no result was recorded.

Patient No. 11 was an example of an RIII response. An 18 year old farmer was admitted with a two day history of headache, muscle pain, fever, and thirst. Admission temperature was 40.4°C. His spleen was not palpable and there were no abnormalities on physical examination. The asexual parasite count on admission was 79,800 per cu.mm. and he was treated with three tablets of Fansidar. By the afternoon of admission, he was in severe distress with headache, abdominal pain, restlessness, and his fever was still 40°C. The parasite count seven hours after treatment had risen to 366,000 and it was decided to infuse 500 mg quinine. A second infusion of 500 mg quinine was given the following morning when the parasite count was 236,000. The patient did not require additional therapy, and attained a radical cure of his malaria. Despite the fact that the elapsed time between treatment with the drug under evaluation and the initiation of quinine therapy was only eight hours, it was felt that this patient represented a failure of the pyrimethamine–sulfadoxine combination in view of his worsening clinical condition and progressive rise in parasitemia.

Toxicity: Two patients developed rashes after the pyrimethamine–sulfadoxine combination which were considered to be related to the administration of the drug. In one patient the rash consisted of giant urticaria, appearing 34 hours after dosing, which resolved after treatment with antihistamines and dexamethasone. Another patient developed a pruritic erythematous rash four days after therapy, which disappeared spontaneously without additional treatment. Neither patient had mucous membrane lesions, and aside from multivitamins, neither had received additional medication. Radical cure was achieved in both patients.

PYRIMETHAMINE 50 MG, DFD 800 MG.

Thirty patients received a single four tablet dose of the combination. Fever clearance time (mean 59 hours) and parasite clearance time (mean 60 hours) were short. However this group of patients had a low mean

initial parasite count (17,000 per cu.mm.), and were, usually, clinically mild cases. Twenty-three patients were followed throughout the 28 day period (Table 2); only ten were cured. Of the failures, two had RIII responses and two exhibited RII responses. Nine had recrudescences of pasraitemia before the end of the 28 day observation period, and were considered RI failures. The over-all cure rate for pyrimethamine-DFD at this dosage was 43%. There was no evidence of hematologic or other toxicity.

The case-history of a patient with an RII response is given below.

Patient No. 50: A 42 year old tailor presented with a four day history of fever, headache, anorexia and vomiting. A cough had been present for six days. He gave a history of malaria 10 years previously. Examination showed a temperature of 40.0°C and rhonchi in the chest. The asexual density of *P. falciparum* was 40,700 per cu.mm. The four tablet dose was administered at 1100 hours. The patient felt better in the evening. By 1400 hours the following day, the parasite density had decreased to 400 per cu.mm. and the patient had a temperature of 38.0°C. He developed fever and chills at 2000 hours, his temperature rose to 39.6°C and his parasite count increased to 10,800 per cu.mm. An RII response was diagnosed and 500 mg quinine was administered intravenously in 500 ml saline over four hours. Altogether nine doses (eight oral) of quinine were given, followed by a single dose of pyrimethamine-sulfadoxine. The patient made a rapid recovery.

PYRIMETHAMINE 25 MG WITH DFD 400 MG, AND PYRIMETHAMINE ALONE.

Early in the study, three patients were treated with the pyrimethamine-DFD combination at this lower dosage (two tablets). Of the three, one responded promptly (S), and two were RII failures. Because of the apparently unacceptable therapeutic action of the combination at this dosage, no further patients were studied.

Three patients with mild illness and low parasitemias were treated with 50 mg pyrimethamine daily for three days. In all three, only a temporary reduction in parasitemia resulted (RII), and symptoms persisted, supporting an earlier impression of resistance of the local strain of *P. falciparum* to this drug.

DISCUSSION: Pyrimethamine 50 mg with DFD 800 mg is only partially effective as an antimalarial, since only 43% of mildly ill patients were cured. This cure rate is similar to that for a pyrimethamine-dapsone (DDS) combination, which was 19% effective (chi square $= 2.0$, $p > 0.05$) (9). DFD has a metabolic half-life of 30 hours, (10) compared with 21 hours for DDS (11). Pyrimethamine, which has a half-life of 96 hours (12), used with either DFD or DDS leads to an unbalanced synergistic combination. Obviously at certain times only an effective dose of pyrimethamine is present in the blood. Since pyrimethamine resistance was demonstrated in this study, the partial therapeutic efficacy of pyrimethamine with DFD may be explicable on this basis. Conversely, pyrimethamine-sulfadoxine is a balanced combination since the half-life of sulfadoxine is 200 hours, (13), and of pyrimethamine about 96 hours.

In this study, pyrimethamine with sulfadoxine cured 85% of patients with an average parasite count of 60,000 per cu.mm., when administered in an adult dose of three tablets (pyrimethamine 75 mg, sulfadoxine 1500 mg.). Thus the combination (Table 3) was twice as effective as pyrimethamine-DFD ($p < 0.01$) in patients whose average parasite count was three times greater ($p < 0.001$).

In Northeast Thailand, a two tablet dose (pyrimethamine 50 mg, sulfadoxine 1000 mg.) cured 82% of patients with an average count of 28,000 per. cu.mm. (9).

Pyrimethamine-sulfadoxine alone is an effective and convenient antimalarial. However, in the opinion of the authors, its major limitation is the somewhat slow control of fever and clinical symptoms (viz patients No. 5 and 11 above), The prolonged activity of the drug, when preceded by the rapid action of quinine, results in the currently optimal therapy for drug-resistant falciparum malaria (14).

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Table 1. Falciparum Malaria in Thailand. Single Dose Therapy with Pyrimethamine 75 mg. and Sulfadoxine 1.5 gm. (Smaller Dose in Children)

Patient Number	Initial Asexual Count *P. falciparum* (per. cc. mm.)	Parasite Clearance Time (Hours)	Initial Fever (°C)	Fever Clearance Time (Hours)	Result
1	314500	—*	39.4	—*	—*
2	286900	—	39.3	—	RIII***
3	258400	—	41.0	—	RIII
4	179600	—	38.5	—	—
5	115900	65	39.2	51	S
6	111000	88	37.0	—	S
7	98000	66	39.9	—	S
8	93300	118	40.4	69	RI
9	92200	—	40.0	67	S
10	90300	76	38.1	68	S
11	79800	—	40.4	—	RIII
12	71300	88	40.3	87	S
13	65300	113	40.2	88	S
14	65000	—	39.7	—	RIII
15	63100	68	39.8	55	S
16	61800	69	40.0	61	S
17	58900	—	39.3	66	S
18	58700	—	40.1	63	S
19	58700	—	40.1	66	S
20	56200	—	39.9	64	S
21	50400	70	40.9	74	S
22	44200	—	39.4	66	S
23	36100**	—	39.8	42	S
24	30400	—	39.6	—	RI
25	27200	76	40.1	68	S
26	26100	62	39.4	37	S
27	24500	—	39.5	64	S
28	23400	66	39.0	41	S
29	21300	75	40.4	83	S
30	19400	63	40.3	62	S
31	17300	61	38.2	84	S
32	17200	—	37.3	—	S
33	16200	—	40.2	66	—
34	13400	—	39.4	—	S
35	10900	117	39.1	37	—
36	10100	93	40.0	109	S
37	9400	39	39.4	—	S
38	8600	—	40.3	—	—
39	6800	—	39.6	35	S
40	6400	70	39.9	57	S
41	4900	—	39.0	42	—
42	4800	45	38.7	44	S
43	3000	52	39.2	—	S
44	2300	—	37.3	—	S
45	1700	38	37.0	—	S
Mean	60300	73	39.5	63	Cure Rate = 85% (33/39)

* If period of observation not adequate, no result given.

** Median count.

*** If no symbol, final result could not be determined.
RIII, no marked reduction of asexual parasitemia;
RII, marked reduction of asexual parasitemia, but no clearance;
RI, clearance of asexual parasitemia, followed by recrudescence;
S, clearance of asexual parasitemia, without recrudescence (radical cure).
World Health Organization (1967) Tech. Rep. Ser. No. 375, p. 42.

Table 2. Falciparum Malaria in Thailand.
Single Dose Therapy with Pyrimethamine 50 mg and DFD 800 mg

Patient Number	Initial Asexual Count *P. falciparum* (Per cu. mm.)	Parasite Clearance Time (Hours)	Initial Fever (°C)	Fever Clearance Time (Hours)	Result
46	95600	93	39.0	84	RI
47	63700	—*	39.2	—*	—*
48	46100	43	39.2	42	—
49	41000	—	38.4	—	RII
50	40700	—	40.0	—	RII
51	30300	69	39.1	69	S
52	20200	44	39.0	50	S
53	18400	117	37.8	—	RI
54	13000	52	40.5	—	—
55	13000	62	38.3	12	—
56	12400	45	37.3	—	S
57	12300	70	40.4	93	RI
58	12200	67	36.9	—	S
59	11700	84	39.8	71	S
60	11200	—	40.5	—	RIII
61	10000**	—	40.4	—	RIII
62	7300	66	37.7	—	—
63	7200	60	39.3	59	RI
64	7200	44	37.1	—	S
65	6900	43	37.8	—	S
66	5700	40	37.0	—	S
67	5400	92	39.2	67	RI
68	4900	72	40.3	40	RI
69	2600	41	38.6	160+	RI
70	1800	61	39.6	12	—
71	1600	45	38.7	37	RI
72	1400	61	38.8	36	S
73	1200	14	39.8	—	S
74	1100	—	40.0	—	—
75	1000	61	38.7	60	RI
Mean	16,900	60	38.9	59	Cure Rate = 43 % (10/23)

* If period of observation not adequate, no result given.
** Median count.

Table 3. Comparison of Cure Rates*

Drug	Average Initial Parasite Count (Per cu.mm.)	RIII	RII	RI	S	Cure*** Rate (%)
Pyrimethamine+ Sulfadoxine	60,300**	4	0	2	33	85 %
Pyrimethamine+ DFD	16,900**	2	2	9	10	43 %

* Patients who did not complete follow-up examinations were not included in this table.

** The average initial parasite counts were significantly different ($t=8.4$, $p<0.001$)

*** The difference in cure rates was statistically significant ($x^2=9.67$, $p<0.01$)

Falciparum Malaria Cured by Quinine Followed by Fansidar (Sulfadoxine with Pyrimethamine)

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OBJECTIVE: To determine the efficacy of a short course of quinine (about three days) followed by a single dose of sulfadoxine with pyrimethamine against falciparum malaria in Thailand.

BACKGROUND: The treatment of chloroquine-resistant falciparum malaria has hitherto been confusing. No clearcut regimen has yet emerged (1). The World Health Organization (2) mentions 16 different regimens involving 12 drugs administered over 1 to 14 days. Based upon the results of this study we recommend one regimen involving three drugs given in about three days.

In Southeast Thailand a six day course of quinine cured 85% of patients with falciparum malaria (3); a single dose of Fansidar (sulfadoxine with pyrimethamine) was also 85% curative (4). Therefore we tested a short course of quinine (average 3 days) followed by Fansidar in about 400 patients.

DESCRIPTION: The Trad Provincial Hospital is in southeast Thailand 400 Km from Bangkok. Malaria is endemic in the area throughout the year. Volunteers were selected for the study if their asexual parasitemia of *P. falciparum* was at least 1,000 per cu.mm. Initially only adult males were studied. When quinine-Fansidar was shown to be the most effective therapy, it was used routinely on adults and children. Patients with coincident vivax malaria were treated but not included in this study.

Quantitative parasite counts (5) were performed at least twice daily in hospital on finger-stick specimens taken at 0800 and 1400 hours and at follow-up examinations on days 14, 21 and 28. Hematocrit levels (packed cell volume) and leukocyte counts were made on admission and whenever clinically indicated.

The drugs were administered by the study physicians during ward rounds usually conducted at 0600, 1400 and 2200 hours. Intravenous quinine was administered as the dihydrochloride salt. The standard dose in adults was 490 mg quinine base in 500 ml normal saline infused over a four hour interval. Oral quinine was prescribed as tablets of quinine sulfate each containing 270 mg base. The routine formulation was a sugar-coated tablet, but plain tablets were occasionally used especially in small children. The standard dose in adults was 540 mg (two tablets) every 8 hours. Each plain tablet of Fansidar (Hoffman-La Roche) contained 25 mg pyrimethamine and 500 mg sulfadoxine. The standard dose in adults was three tablets given with or eight hours after the last dose of quinine.

Quinine concentrations on random sera were determined by the benzene extraction method of Brodie and Udenfriend (6). This is an accurate technique (7).

Parasite and fever clearance times were determined in hours. The patients' temperature charts were retained for analysis. Patients were considered radically cured (S response) if the parasitemia was cleared by treatment and had not reappeared before day 29 (the day of admission being called day 0). The WHO (8) classification was used (Table 2).

PROGRESS: Four hundred and fourteen patients were admitted to the study. Nine patients left the hospital after clinical improvement but before receiving Fansidar. Therefore 392 patients received

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1 Trad Provincial Hospital, Thailand

quinine—Fansidar therapy. The average age of the group was 23.9 years (range 1—73) and average weight 45.4 Kg (range 5—80). Only 14 patients were female since we usually confined our studies to male patients.

Most patients were fairly seriously ill on admission. The average parasite count (90,676 per cu.mm.) was much higher than in our other therapeutic studies (3) and the average parasite clearance time of 77.3 hours indicates a satisfactory response (Table 1). Clearance of parasitemia was slow in a few patients, especially those with high parasite counts (e.g. over 200,000 per cu.mm.) and was possibly due to partial resistance to quinine. A typical patient was No. 391 with a parasite count of 252,720 per cu.mm. He received 18 doses (six days) of quinine (Table 1) before the Fansidar. His parasite clearance time was prolonged at 147 hours. Despite the slow clinical response, his blood was free of parasites on days 14, 21 and 28 and so he was accredited with a radical cure.

The average fever clearance time was 61.5 hours. In a few patients a persistent fever was probably caused by the quinine. Since the average course of quinine was short; quinine fever was not so frequent a problem as with longer courses in previous studies (9). In some patients, who received only a few doses of quinine, a rising temperature on day 1 or day 2 was probably caused by the Fansidar, since the fever patterns were similar to those found in patients receiving Fansidar alone (4). Many patients were discharged when they felt better but before the fever and parasitemia had cleared.

Eighty two percent (322/392) of the patients received at least one dose (average 1.5 doses, range 1—7) of quinine as a continuous intravenous infusion. The standard dose of quinine base was 10 mg per Kg but in small children a lower dose was often used to prevent toxicity. The adult intravenous dose (490 mg quinine base in the dihydrochloride salt) was usually given in 500 ml normal saline. The optimum infusion time for a rapid response and avoidance of toxicity was four hours. Half strength solutions (0.5 mg quinine base per ml) were administered, especially in children, if quinine toxicity was suspected. In comatose adults optimum therapy was not more than 1,000 mg quinine base (two doses) per 24 hour interval given in 1000—1500 ml fluid intravenously. Quinine metabolism is impaired in severe falciparum malaria and the half—life of the drug is prolonged. Thus lower or less frequent doses are needed to prevent overdosage. In several of our patients toxic levels of serum quinine (concentration over 10 mg/L) occurred despite subnormal doses of the drug. Nevertheless quinine is the only drug that brings severe falciparum infections under satisfactory control in Southeast Thailand.

Follow—up examinations were completed in 314 patients, of whom 302 (96%) were radically cured (Table 2). The quinine—Fansidar regimen was more effective than a six day course of quinine, or Fansidar alone, in our test system.

Initially all patients received nine doses of quinine. The questions arose whether a shorter course of quinine would be equally effective and whether, in severe cases, a longer course of quinine would be more effective. Therefore, additional patients received from 1 to 18 doses of quinine before the single dose of Fansidar. To our surprise the cure—rate was about 96% throughout the range (Table 1). However the cure—rate of 98% (43/44) with 10 to 18 doses was impressive since the patients were mostly seriously ill and their average parasitemia was high (166,000 per cu.mm.).

If the course of quinine was very short (less than four doses), then the initial clinical improvement was not always maintained and a temporary resurgence of fever and other symptoms often occurred on about the second day. If at least four doses of quinine were given, optimal clinical improvement usually resulted. Table 1 shows that the shortest fever clearance time occurred with the four dose course. Longer courses of quinine appeared to be indicated in patients with high initial parasite counts or evidence of chronic disease (e.g. large spleens).

Quinine caused typical mild side—effects in most patients (e.g. nausea and tinnitus). If blurred vision or other serious symptoms occurred, the dose of quinine was reduced. Serious side—effects were more frequent in children than in adults (e.g. coma, convulsions). Reduction or deletion of dosage usually resulted in a rapid decrease in toxicity.

Serious toxicity attributable to the Fansidar did not occur in any of the 392 patients who received the the quinine—Fansidar regimen. However Fansidar as solo therapy often causes fever and less often urticarial rashes (4).

The cost of treatment comprises the hospital costs (if the patient requires admission) plus the cost of the drugs. The 392 patients were in hospital for an average of only 3.8 days which was brief in relation to the average clinical severity of the group, and reflects the fact that quinine usually acts rapidly.

We determined that the minimal effective regimen was four doses of quinine plus Fansidar. At the time of the study quinine cost the hospital pharmacy about U.S. $0.04 per tablet and Fansidar about $0.15 a tablet. Thus the cost of the regimen orally was at least $0.77. This was less expensive than any other effective regimen. Intravenous therapy was much more expensive because units of intravenous fluid were relatively costly.

Other relevant factors are the duration of therapy, the total number of doses and the frequency of dosing. Optimal quinine—Fansidar therapy comprises at least five doses (four quinine, one Fansidar) given over two days at eight hour intervals. These parameters are less than for any other effective regimen.

DISCUSSION: This study has established that quinine followed by Fansidar is the treatment of choice for chloroquine—resistant falciparum malaria. The regimen is theoretically sound because it comprises the rapidly acting drug quinine, followed by the long acting combination of sulfadoxine and pyrimethamine (Fansidar). The quinine brings the infection under control and the Fansidar assists in its eradication. This regimen is useful because we found that Fansidar is non—toxic when given at the end of a course of quinine; it is practical because it is completed in about three days, thus allowing the patient to be discharged promptly.

The value of an antimalarial regimen may be determined by five criteria viz., efficacy, toxicity, cost, duration of therapy and length of hospital stay. These criteria must be judged in relation to the average severity of the cases being treated, which is indicated by the clinical severity and the average parasite count. Considering all these factors, the quinine—Fansidar regimen is the best that we have tested.

The components of the quinine—Fansidar regimen are more powerful antimalarials than any alternative drugs. For chloroquine—resistant falciparum malaria, quinine is the only rapidly acting drug currently available. The components of Fansidar are longer acting than any other similar drugs (Table 3). Fansidar is more effective in the radical cure of *P. falciparum* than pyrimethamine with DDS (10) or pyrimethamine with DFD (4). Single dose Fansidar is more effective than multi—dose clindamycin or tetracycline (11). It should be stressed that Fansidar alone is often toxic (e.g. fever, urticaria) and slow acting, but when given at the end of a course of quinine, it caused no serious toxicity in over 300 patients. Sequential quinine—Fansidar is also more logical than combination therapy because antimalarial activity in the blood is obviously maintained for a longer period.

Quinine has, of course, been used for malaria for several hundred years. Sulfadoxine was introduced for malaria by Laing (12). Chin (13) discovered the antimalarial potentiation of sulfadoxine and pyrimethamine. The U.S. Army found that a 14 day course of quinine with an initial dose of sulfadoxine and pyrimethamine was effective against falciparum malaria (14, 15). However the official U.S. Army regimen is still quinine for 10 days, pyrimethamine for 3 days and DDS for 29 days (16). In Laos a 7—10 day course of quinine plus an initial dose of Fansidar was found to be effective (17). However quinine with Fansidar is not mentioned in the regimens recommended by W.H.O. (2) nor in the latest addition of a leading textbook of medicine (18).

There is evidence that quinine is more effective than chloroquine against chlorquine–sensitive falciparum malaria from Africa (19). Therefore comparative studies of quinine and chloroquine with and without terminal Fansidar for African falciparum are obviously indicated.

RECOMMENDATIONS: The following is the recommended treatment of falciparum malaria in Thailand based on all completely tested drug regimens to date:

1. At least four doses of quinine (540 mg base each dose in adults) usually given at 12 hour intervals followed by a single dose of Fansidar (sulfadoxine 1.5 g with pyrimethamine 75 mg in adults). Proportionately smaller doses are given to children.
2. Quinine dosage should not exceed 20 mg/kg daily. The first and sometimes subsequent doses of quinine should often be administered as an intravenous infusion usually in four hours. The standard dose in adults is 500 mg (10 gr) in 500 ml normal saline.
3. In order to prevent pulmonary edema, Thai adults with falciparum malaria should not receive more than 1500 ml fluid (including blood transfusion) every 24 hours. Children should receive proportionately smaller volumes.
4. Blood transfusion is rarely indicated in falciparum malaria and antimalarial therapy is usually sufficient. Blood transfusion should be considered if the hematocrit falls below 15%.

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Table 1. Relation of the Number of Quinine Doses to the Falciparum Malaria Cure Rate.

No. Doses Quinine	Average Parasite Count For Group	Average Parasite Clearance Time (Hrs)	Average Initial Fever (°C)	Average Fever Clearance Time (Hrs)	Treatment Failures (RI–III)	Cure (S)	Cure Rate
0*	60230	73.0	39.5	62.6	6	33	85 %
1	57742	67.3	39.1	50.7	0	21	
2	65510	71.6	39.5	57.5	2	22	
3	71719	67.7	39.3	55.6	0	24	
4**	124624	72.4	39.4	43.4	0	22	
5	76385	67.4	39.5	54.5	2	22	
6	81481	73.8	38.8	62.5	1	21	
7	114285	75.3	39.4	58.0	0	23	
8	99600	78.2	39.3	70.3	1	21	
9	64067	76.6	39.2	59.5	5	83	
10	98651	81.5	38.5	69.5	0	20	
11	66949	91.8	39.6	102.0	0	7	
12	285057	88.0	39.0	74.8	1	6	
13	242345	92.5	39.2	93.5	0	3	
14	269754	108.8	38.7	91.0	0	3	
15	138340	117.0	40.3	109.5	0	2	
16	—	—	—	—	—	—	
17	515060	130.0	40.1	82.0	0	1	
18	252720	147.0	40.2	137.0	0	1	
Average 1–18 Doses (Total Quinine-Fansidar Group)	90676	77.3	39.2	61.5	12	320	96 %

* Study reported in detail elsewhere (4)

** For optimum clinical response at least 4 doses of quinine should be given before the Fansidar.

Table 2. Falciparum Malaria in Thailand 1973–1974.
- Cure Rates with 4 Different Regimens.

Drug Regimen	Average Duration Therapy	Average Parasitemia (per cu.mm.)	RIII*	RII	RI	S	Cure Rate (%)
Quinine+Fansidar	3 days	90,000	0	0	12	302	96
Quinine**	6 days	28,000	1	0	9	55	85
Fansidar***	1 dose	56,000	4	0	2	33	85
Chloroquine	3 days	15,000	2	8	1	0	0

* RIII, no marked reduction of asexual parasitemia; RII, marked reduction of asexual parasitemia, but no clearance; RI, clearance of asexual parasitemia, followed by recrudescence; S, clearance of asexual parasitemia, without recrudescence (radical cure). World Health Organization (1967).
** Study reported in detail in Hall, A.P. et al. [3]
*** Dose in adults 1.5 g sulfadoxine and 75 mg pyrimethamine.

Table 3. Dihydrofolate Reductase Inhibitors, Sulphonamides and Sulphones Used in Malaria – Half-life (t/2)

Drug	Half-life (T/2 in Hours)	Reference
Sulfadoxine*	200	Brooks, M.H. et al. (15)
Pyrimethamine*	96	Smith, C.C., and Ihrig, J. (20)
Sulfalene	65	Seneca, H. (21)
DFD	30	Sonntag, A.C, et al. (22)
DDS	21	Glazko, A.J. et al. (23)
Sulfadiazine	17	Richards, W.H.G. (24)
Sulfamethoxazole**	9	Schwartz, D.E., and Rieder, J. (25)
Trimethoprim**	9	Schwartz, D.E., and Rieder, J. (25)

* Sulfadoxine with pyrimethamine is marketed as Fansidar.
** Sulfamethoxazole with trimethoprim (co-trimoxazole) is marketed as Bactrim or Septrin.

Comparison of Mefloquine (WR 142490) and Pyrimethamine with Sulfadoxine for the Single-Dose Treatment of Falciparum Malaria

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OBJECTIVE: To compare the efficacy of mefloquine and Fansidar in the treatment of falciparum malaria.

BACKGROUND: Mefloquine (WR 142490) is α – (2–piperidyl)–2, 8–bis (trifluoromethyl)–4–quinoline methanol hydrochloride. Mefloquine, given as a single dose, has been very effective in the treatment of induced falciparum malaria in prison volunteers in the United States (1). It is a long acting chemical analogue of WR 30090. In Thailand WR 30090 was as effective as quinine when administered every 8 hours for 6 days in the treatment of falciparum malaria.

Fansidar (a 20:1 combination of sulfadoxine and pyrimethamine) has been extensively studied as a single dose for the treatment of falciparum malaria. In Thailand Fansidar was 85% curative in Trad Province and 82% curative in Prachinburi Province in the SEATO studies.

DESCRIPTION: The study was begun at the Chao Phya Abhai Bhu Bejhr (Prachinburi Provincial Hospital) on 24 February 1975. Male patients who volunteered were selected for study if they were aged at least 15 years. Other criteria were an asexual parasite density of *P. falciparum* of at least 1,000 per cu.mm. and the ability of the patient to return for follow-up examination on days 14, 21 and 28 after therapy. Also the patients were asked to sign a written consent after being informed of the nature and potential hazards of the study. Thirty patients will be treated in each group.

Direct estimations of the parasite density were made on admission, twice daily in hospital and once at follow-up examination on days 14, 21 and 28. Determinations of the hematocrit and WBC count were made daily in hospital and at the follow-up examinations. Urinalysis was performed on admission and whenever subsequently indicated.

The medications were administered by the nursing staff in the presence of a study physician. Mefloquine was supplied as plain tablets each containing 250 mg of the drug. The dose was 1.5 g (six tablets).

Sulfadoxine–pyrimethamine was prescribed as plain tablets each containing 500 mg sulfadoxine and 25 mg pyrimethamine. The dose was three tablets.

PROGRESS: *MEFLOQUINE* acted more quickly against falciparum malaria than did Fansidar (Tables 1 and 2). However, even with mefloquine, the patients' symptoms did not always respond as quickly as the parasite and fever clearance time would suggest.

The parasite clearance time for mefloquine was 59 hours which is shorter than for the 12 other regimens that we have tested. For example, the parasite clearance time for quinine, in a group of patients of similar clinical severity, was 69 hours. The average fever clearance time for mefloquine was 46 hours.

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Only amodiaquine had a shorter fever clearance time (36 hours). In most patients who received mefloquine, fever cleared rapidly, but in two it was more prolonged (Cases 3 and 5).

One patient showed clinical deterioration during the first few hours after the dose of mefloquine and an RIII failure was diagnosed. The patient responded to quinine and the case history is given below. Follow-up has been completed on nine other patients and all achieved radical cures of their infection.

Patient No. 1. was an 18 year old laborer with a history of headache and fever for five days. He complained of a bitter taste in his mouth but was not thirsty. He was afebrile on admission (temperature 37.2°C) and the pulse rate was 90 per minute. The blood pressure was 90/50. He looked slightly anemic but was not jaundiced. His abdomen was soft and the liver and spleen were both moderately enlarged. He could stand and stagger about but he preferred to lie down. He was alert and signed the consent form. His skin was cool without perspiration. The initial parasite density (5 minute stain) was 96,000 per cu.mm. He was diagnosed as having moderately severe falciparum malaria. Because he was afebrile and hypotensive, the old term "algid malaria" was probably appropriate. The hypotension was probably due to salt depletion or possibly dehydration. Mefloquine 1.5 g orally was administered at 0945 hours and 500 ml normal saline was infused over a four hour interval. The Accurate Count (30 minute stain) was 171,000 per cu. mm. At 1330 hours the parasite count was 208,000 per cu.mm., but the patient had a large lunch. At 1600 hours the patient sat on the floor (as was his custom) and had a large meal. He then developed severe abdominal pain. On examination the patient was groaning and writhing in agony. An infusion of quinine (500 mg in 500 ml normal saline) was begun at 1650 hours and was infused in 3.5 hours. The serum quinine concentration increased from 0 to 13.3 mg per liter during the infusion.

The severe abdominal pain disappeared within 30 minutes of the beginning of the infusion and overall the patient greatly improved. The parasite density, however, was 407,000 per cu.mm. at 1745 hours and 468,000 at 2020 hours. A fever of 39.1°C developed during the day.

The patient's condition was satisfactory the next morning but the parasite density was still 450,000. per cu.mm. A second infusion of quinine 500 mg in 500 ml normal saline was infused over a four hour interval. At the end the patient had tinnitus indicating quinine toxicity. However at 1300 hours the parasite density had decreased to 141,000 per cu.mm. A third and smaller dose of quinine (270 mg orally) was given at 1800 hours. Thereafter the patient made a steady recovery and remained free of parasitemia on days 14, 21 and 28,

The parasitemia cleared in 99 hours and the fever in 88 hours. During the initial 24 hours the hematocrit (packed cell volume) decreased from 40 to 26 per cent. Thereafter, coincident with the eradication of his disease, the hematocrit increased to 41 per cent on day 28 without any hematinic therapy.

FANSIDAR: The single dose combination of pyrimethamine with sulfadoxine (Fansidar) cleared parasitemia on average more slowly (76 hours) than did mefloquine (59 hours). Clinically Fansidar acted slowly in many patients (case histories given below). The fever clearance time (62 hours) was longer than that for mefloquine (46 hours). The differences between the clearance times are not statistically significant but probably will be when more patients have been studied. So far, 6 out of 8 patients have been cured.

Patient No. 16. This 35 year old laborer had headache and myalgia for four days. There was no history of previous malaria. His temperature was 40.0°C, pulse rate 90, blood pressure 120/80 and weight 46 kg. He appeared mildly jaundiced but not anemic. He was alert but tired and could only walk with assistance. The parasite density was 100,000 on the slide stained for five minutes and 78,000 on the 30 minute slide. The dose of Fansidar was given at 1530 hours. Because his lips were dry, dehydration was diagnosed. One thousand milliliters 5% dextrose in saline were infused over a four hour interval. On day 1 the parasite density decreased to 12,000 but his fever resurged to 40.5°C on day 1 and to 41.0°C on day 2. The patient was observed carefully and he improved; however, anorexia persisted. His parasitemia cleared on the morning of day 6 (135 hours) but reappeared in the afternoon.

Therefore a treatment failure was diagnosed, either RI or RII. The patient received four doses of oral quinine and the parasitemia cleared. He was discharged. On day 28, the patient returned with a parasite count of 1000. Again he received four doses of quinine. On day 42 the patient returned with a count of 2,000. Six days of oral quinine therapy was prescribed as an outpatient. One week later the patient was free of parasitemia.

Patient No. 20. This 36 year old farmer had a history of headache and fever for three days. His temperature was 39.5°C and pulse rate 90 per minute. He was walking and alert. The liver and spleen were not palpable. The parasite density was 31,000 per cu.mm. Fansidar was given at 1730. The parasitemia decreased to 20 per cu.mm. by the afternoon of day 2, at which time the fever resurged to 39.9°C. On day 3 the patient felt better and the fever decreased to 37.8°C at 0600 hours. During the day the parasitemia increased from 20 (overnight) to 900 to 2200 per cu.mm. An RII response was diagnosed. One dose of oral quinine was given at 0900 hours. The parasitemia decreased to 0 by day 6; however, the patient developed a persistent headache and a parasite count of 10 per cu.mm. was again noted on day 9. A six dose course of quinine was now given and another dose of Fansidar. The patient was cured.

The therapeutic result in this patient is somewhat difficult to assess. On day 3 the parasite count increased but the fever decreased and the patient felt better. It could be argued that the quinine was given prematurely. However despite one dose of quinine, the parasitemia returned on day 9. The patient therefore had either an RII or an RI response.

Patient No. 22. This 38 year old laborer had received two shots (probably an antipyretic) two days previously. He did have a history of malaria eight years ago. His temperature was 38.6°C, pulse 104, blood pressure 90/60 and weight 55 kg. The spleen was enlarged. The parasite count was 13,000 per cu.mm. The dose of Fansidar was administered at 1115 hours. At 1300 hours the parasite count was 62,000 but the patient had improved clinically. At 1930 the parasite count had increased slightly to 90,000. The patient felt better and the temperature had decreased from 40.2°C. to 37.2°C. Because of the increase in parasitemia, quinine therapy was begun. The fever cleared in 63 hours. The patient received oral quinine every 12 hours, but the parasitemia persisted at 10–60 per cu.mm. for three days. Therefore the quinine dosing was increased to every 8 hours. The patient finally received 18 doses of quinine. It was later decided that quinine therapy had been instituted prematurely on day 0, so no therapeutic result could be recorded.

DISCUSSION: Fansidar (a 20:1 combination of sulfadoxine with pyrimethamine) has been extensively studied both for the treatment and prevention of malaria. At Prachinburi Hospital in Northeast Thailand in 1972, a single dose of Fansidar cured 82% of a group of patients with falciparum malaria; in Southeast Thailand in 1974 the cure rate was 85%. However it is well known that Fansidar is often slow to bring an infection under control and some infections are resistant to the drug. The preliminary results of this study suggest that a lower cure rate will be found at Prachinburi Hospital in 1975.

In the initial group of patients, mefloquine cleared parasitemia and fever more quickly than did sulfadoxine with pyrimethamine. Clinically mefloquine acted more quickly and appears to be a very effective antimalarial drug; however quinine by intravenous infusion is still required for severe infections. The current recommended treatment for chloroquine resistant falciparum malaria is a course of quinine followed by a single dose of sulfadoxine with pyrimethamine. Mefloquine can be considered a superior substitute for Fansidar in this regimen. A short course (e.g. 2–6 doses) of quinine will probably usually suffice in severe cases and in mildly ill patients, the mefloquine can be given alone.

SUMMARY: Mefloquine 1.5 g (a 4–quinoline methanol) was compared with pyrimethamine 75 mg and sulfadoxine (Fansidar) 1500 mg for the single dose treatment of falciparum malaria. The study is still in progress. So far, mefloquine has cured 90% (9/10) of patients; the average parasite clearance time has

been 59 hours and the average fever clearance time 46 hours. With Fansidar the cure rate has been 75% (6/8), the parasite clearance time 76 hours and the fever clearance time 62 hours.

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Table 1. Falciparum Malaria Treated with Mefloquine (WR 142490)

Patient Number	Initial Asexual Count *P. falciparum* (per cu.mm.)	Parasite Clearance Time (Hours)	Initial Fever (°C)	Fever Clearance Time (Hours)	Result*
1	171000	—	37.2	—	RIII
2	130000	62	38.2	28	
3	68000	67	39.0	46	S
4	28000	68	40.0	79	S
5	27000	44	38.8	31	
6	16000	43	39.8	10	S
7	15000	62	39.1	101	S
8	11000	88	39.8	38	S
9	8000	91	40.4	104	
10	7000	76	38.5	61	S
11	5000	66	39.6	32	S
12	4000	22	39.2	15	S
13	2000	19	39.4	10	S
Mean	38,000	59	39.2	46	

* In no result given, follow-up had not yet been completed.

Table 2. Falciparum Malaria Treated with Fansidar
(Pyrimethamine with Sulfadoxine)

Patient Number	Initial Asexual Count *P. falciparum* (per cu.mm.)	Parasite Clearance Time (Hours)	Initial Fever (°C)	Fever Clearance Time (Hours)	Result
14	104000	91	40.1	110	S
15	81000	—	39.7	—	
16	78000	135	40.0	114	RI
17	56000	73	41.0	104	
18	41000	86	40.5	37	S
19	35000	73	40.0	52	
20	31000	—	39.5	—	RII
21	15000	87	39.9	61	
22	13000	—	40.2	—	See Text
23	13000	67	36.5	—	
24	7000	71	40.0	40	
25	6000	90	37.5	—	S
26	5000	69	39.0	64	
27	4000	88	38.0	104	S
28	4000	67	40.2	58	
29	4000	64	39.6	47	
30	3000	43	38.0	17	S
31	2000	42	38.5	13	
32	1000	68	39.0	43	S
Mean	26,000	76	39.3	62	

The Management of Coma in Falciparum Malaria

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OBJECTIVE: To establish the optimum management of coma in falciparum malaria.

BACKGROUND: Cerebral malaria is common in falciparum malaria and usually occurs in patients with high parasite counts; however, occasionally, patients present in coma with low parasite counts. Coma is the most serious manifestation of cerebral malaria in adults. Epilepsy is also a grave complication especially in small children. Other forms of cerebral malaria include delirium, confusional states and stupor. Often the patients enter hospital seriously ill and stuporous and lapse into coma soon afterwards or after therapy. Often the patients appear to lapse into coma or to come out of coma following minimal therapy. In some patients, therefore, the cerebral malaria is short-lived, whereas in others coma is deep and irreversible.

Quinine is the most effective drug in therapy. Corticosteroids were first recommended in 1967 but have never been subjected to a controlled clinical trial. The anticoagulant heparin has been recommended because some investigators consider disseminated intravascular coagulation to be a common complication of severe falciparum malaria. The plasma volume expander, Dextran, has also been recommended for falciparum coma as has the osmotic diuretic, Mannitol.

DESCRIPTION: Between January 1973 and July 1974 at Trad Provincial Hospital about 40 patients with the various types of cerebral malaria were treated. Further experience has been gained at the Prachinburi Hospital since the project was initiated there in February 1975. The clinical evaluation of the patients has consisted of close observation by the study physicians. Detailed clinical notes have been maintained on specific study sheets. The rate of intravenous therapy has been monitored at regular intervals, usually every 30 minutes. The state of consciousness, pulse rate, and blood pressure were also regularly monitored when indicated. Complete physical examination was regularly performed. If bladder distension occurred, a urethral catheter was passed and the bladder continuously drained into a measured bottle. The rate of urine production was recorded at frequent intervals. Laboratory investigations included a parasite count at least twice daily and a daily hematocrit. Urinalysis was performed on admission and whenever subsequently indicated. Serum was taken for biochemical analysis on admission and at regular intervals thereafter. Relevant investigations were performed a few weeks later at the SEATO laboratory in Bangkok and usually comprised a total serum bilirubin, serum creatinine and serum glutamic oxaloacetic transaminase (SGOT). A serum alkaline phosphatase was also occasionally determined. Serial serum quinine concentrations were determined on most patients. The technique consisted of extraction of the quinine with benzene followed by sulfuric acid and estimation of the fluorescence of the quinine in a spectrofluorophotometer.

PROGRESS: Three case histories are given to illustrate the successful management of cerebral malaria.

Case 1: A 42 year old man (a tree worker) was brought to Trad Hospital at 2000 hours on 11 May 1974. He had been in coma since 1700 hours. The history was of vomiting and anorexia for three days. He consulted a physician in Trad city at midday on the day of admission and a 500 ml unit of intravenous fluid was administered as well as one tablet orally. The patient went home and then lapsed

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2 Trad Provincial Hospital

into coma. He gave a history of malaria two years previously. His temperature was 38.5°C and pulse rate 120. The parasite count was 62,000 per cu.mm. He was in coma but reacted to pain. He was sweating profusely. The obvious question arose as to whether the patient had received quinine in the private clinic earlier in the day. For this reason he was given a half dose of quinine (250 mg) in 500 ml normal saline, infused over five hours. The next morning, 10 hours later, he was awake. A half-dose of quinine was given orally (270 mg) and eight hours later a full dose (540 mg). A full dose of Fansidar (three tablets) was administered in the evening. The patient made a satisfactory recovery and his parasitemia cleared in 83 hours.

His sera were analyzed two weeks later. Surprisingly, the serum was free of quinine on admission. On the morning after receiving the half-dose of intravenous quinine his serum quinine level was 4.2 mg. per L.

Case 2: A 21 year old farmer was admitted in a stuporous condition. His parasite count was 250,000 per cu.mm. but his vital signs were satisfactory. We decided to treat him with intravenous quinine in the standard 500 mg doses in 500 ml normal saline—at 12 hour intervals. The first dose was given in two hours. The serum quinine was 9.0 mg per L at the end of the infusion (Figure 1) and had decreased to 3.6 mg. per L at hour 17 when the second infusion was commenced. This was infused in three hours and raised the serum quinine to 9.3 mg per L. The third infusion was begun at hour 24 for four hours and increased the serum quinine to 10.3 mg per L. The final infusion was begun at hour 38 and infused in five hours. The serum quinine then peaked at 11.0 mg per L. No further quinine was given until hour 71 when three doses of oral quinine were administered over a 15 hour interval. A dose of Fansidar (three tablets) was administered at hour 98. The patient's parasitemia remained high until hour 38 when a steady decrease began. The patient became completely conscious about hour 43. Another notable feature of this case was that the patient had evidence of a bleeding disorder on admission. He had a large amount of recently dried blood in his nostrils and bleeding about venipuncture sites. These signs rapidly cleared with the successful management of his disease.

Case 3: This patient represented a very successful therapeutic result because he appeared moribund on admission. He was in deep coma and flaccid. His respiratory rate was increased at 40 per minute and his heart rate was 130 per minute. There was a loud systolic murmur. There was bleeding about venipuncture sites. The Thai physician gave the relatives a very serious prognosis. The initial parasite count was about 95,000 per cu.mm. The patient's progress can be seen in Figure 2. Doses of intravenous quinine were administered at 0, 16 and 23 hours following admission The patient's consciousness returned towards the end of the third infusion. He received a dose of oral quinine on hour 20 and another dose of intravenous quinine at hour 49. A dose of Fansidar (three tablets) was administered at hour 62. During the first 50 hours the serum quinine concentration remained in the 4—12 mg per L range. The patient made an uneventful recovery.

DISCUSSION: A system for managing severe falciparum malaria has been established by our investigations. Most patients recover with quinine therapy alone but it is important to avoid overdosage. Twenty milligrams per kilogram daily is the maximum safe dose and this is administered intravenously not more frequently than every 12 hours. As discussed elsewhere, fluid input should be restricted to prevent pulmonary edema. Corticosteroid therapy has not been discussed in this paper and its value is difficult to determine.

Pulmonary Edema Due to Fluid Overload in Falciparum Malaria

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OBJECTIVE: To determine whether restriction of intravenous fluid intake would decrease the incidence of pulmonary edema in comatose patients with falciparum malaria.

BACKGROUND. Brooks et al. presented case-histories on five patients with falciparum malaria who developed pulmonary edema and died (1). Four of the patients had received intravenous fluids. Punyagupta et al. described 12 patients with this complication of whom nine died (2). All patients had received intravenous fluids. Both authors claim that the pulmonary edema was not due to fluid overload but to a specific complication of the disease.

This paper reports on six comatose adult patients with falciparum malaria who developed pulmonary edema which was attributed to excessive intravenous fluid therapy. Ten other comatose patients with falciparum malaria, studied later, did not develop pulmonary edema and this was attributed to optimal intravenous fluid therapy.

DESCRIPTION: The study was carried out at the Trad Provincial Hospital in Southeastern Thailand 400 km from Bangkok. The area is endemic for chloroquine-resistant falciparum malaria (3, 4). Diagnostic services and nursing facilities were limited at the time of the study (1973-1974). Study physicians maintained detailed clinical records, closely monitored intravenous fluid therapy and, in most patients, recorded the approximate urine output. The patients were seriously ill on admission and there was no facility for weighing them in bed before treatment. Central venous pressures (CVP) could not be monitored.

Initially eight comatose patients (six adults, two children) with falciparum malaria and pulmonary edema were studied. Not all patients were under the direct care of the research physicians. Following the restriction of intravenous fluid intake as a change in therapeutic policy, 10 comatose adults with falciparum malaria were studied who did not develop pulmonary edema. The two groups were equivalent with respect to clinical severity and average parasite densities.

Quantitative parasite counts (5) were determined at least twice daily in hospital and at follow-up examinations on days 14, 21 and 28 and often later. Hematocrits and white blood cell counts were performed regularly. Sera were collected and taken to the SEATO Laboratory in Bangkok for determination of quinine concentrations (6). There were no facilities at Trad for radiography or autopsies.

PROGRESS: In six comatose adults the onset of pulmonary edema was apparently related to intravenous fluid therapy (Table 1). These patients received an average of 1,767 ml intravenous fluid during the first eight hours after admission and altogether 2,825 ml in the first 24 hours. The physical signs of pulmonary edema developed following intravenous therapy. In all six patients, fluid intake greatly exceeded urine output during the period that pulmonary edema developed. In three patients, coma developed or worsened after intravenous fluids.

Ten comatose adult patients studied later did not develop pulmonary edema. Their average fluid intake was 563 ml in the first eight hours and 1,181 ml in the first 24 hours (Table 2). The differences between the eight hour inputs ($t = 4.2$, $p < 0.001$) and 24 hour inputs ($t = 4.1$, $p < 0.001$) were statistically significant. No clinical deterioration followed intravenous therapy in the second group.

1 Trad Provincial Hospital

The case-histories on two adults and two children are now given who were not under the care of physicians from SEATO Medical Research Laboratory.

Case No. 2: This 30 year old woman was six months pregnant. She had become comatose on the day of admission. On examination the lung-fields were clear. Her temperature was 38.8°C and her asexual parasite density of *P. falciparum* was 94,600 per cu.mm. Since she was pregnant, quinine was not prescribed for fear of abortion. One thousand milliliters of five per cent dextrose with 200 mg chloroquine base was infused in one hour. A second similar unit was infused in the following six hours. Thus within eight hours of admission the patient had received 2,000 ml fluid. The patient developed noisy breathing. Loud moist crepitations were present throughout both lung-fields. Since her falciparum malaria was deemed resistant to chloroquine, a third liter with 1,000 mg quinine was now administered. The next morning she awoke briefly but lapsed into coma following further intravenous fluids which were administered at the rate of 1,000 ml every eight hours. The physical signs of gross pulmonary edema persisted and she died 24 hours later at which times the parasite count had decreased to 60 per cu.mm.

Case No. 6: This 18 year old man had been ill for eight days and aphasic for two days. The patient had been taken to an unlicensed practitioner on the day before admission. Four thousand milliliters of intravenous fluid were infused by the practitioner over a 12 hour interval. After 3,000 ml, the patient was able to walk to the toilet (according to his brother). Another 1,000 ml was rapidly infused and the patient went into a deep coma from which he never recovered. He was brought into the hospital moribund about 10 hours later, coughing up blood-tinged sputum. He had cyanosis and was breathing noisily. Extensive moist râles were heard. The *P. falciparum* density was 130,000 per cu.mm. In hospital he received quinine 500 mg in 500 ml normal saline intravenously over a four hour interval. The diuretic, furosemide 20 mg, was administered intravenously and urine output was 800 ml during the next 12 hours. A second quinine infusion was begun 16 hours after admission. The total fluid input in hospital was 700 ml and output 1,300 ml, but the physical signs and gross pulmonary edema worsened. The patient deteriorated and died 22 hours after admission.

Case No 17: This four year old boy weighed 10 kg. On admission at 1100 hours he was stuporous with enlarged liver and spleen. The *P. falciparum* density was 273,000 per cu.mm. Quinine 250 mg in 500 ml five per cent dextrose in half-normal saline was infused over five hours. His condition deteriorated and signs of pulmonary edema supervened. On the next day he was in coma and extensive bubbly râles were heard over both lung-fields. The parasite count had decreased to 48,000 per cu.mm. Another 250 mg quinine in 500 ml was infused in eight hours. After two hours his coma had deepened and spleen had become larger. Convulsions appeared, which were only partly responsive to Nembutal intramuscularly and intravenously. He developed an extension spasm of the neck. On the next day his parasite count had decreased to 10,000 per cu.mm. The physical signs of severe pulmonary edema persisted. Another 250 mg quinine in 500 ml was infused during the day and the parasite count decreased to 2,000 per cu.mm. The boy was moribund and his father took him home to die.

Comment: In this 10 kg boy, an initial 500 ml infusion appeared to precipitate pulmonary edema.

Case No. 18: This five year old girl weighing 12 kg was admitted seriously ill but fully alert. Her asexual count of *P. falciparum* was 396,000 per cu.mm. Quinine 250 mg in 250 ml normal saline was infused over 90 minutes. Her lungs remained clear at the end of the infusion. On the next morning, 17 hours after admission, her condition appeared to have improved. The parasite count had decreased to 54,000 per cu.mm. Another 250 mg quinine was infused in 500 ml and she developed fits and went into coma. Extensive râles were audible throughout both lung-fields. Twenty-four hours later another infusion of 500 ml quinine was given. She continued to deteriorate with the clinical signs of coma and gross pulmonary edema and died.

Comment: In this 12 kg girl, an initial infusion of 250 ml was uneventful but a subsequent infusion of 500 ml precipitated a fatal pulmonary edema.

OPTIMAL INTRAVENOUS HYDRATION: As described above, pulmonary edema developed in eight patients (six adults, two children) following intravenous fluid. Therefore in subsequent patients fluid input was restricted.

The patients were in coma on admission or lapsed into coma shortly afterwards. None developed pulmonary edema and this was attributed to the deliberate restriction of intravenous therapy (Table 2). A positive fluid balance was not detected in any patients. The following case description is representative.

Case No. 12: This 26 year old man weighed 45 kg and was admitted in coma. His initial asexual count of *P. falciparum* was 10,800 per cu.mm. which cleared within 64 hours. Intravenous fluid intake over the first four days was 1,000, 1,000, 1,450 and 1,000 ml, respectively. On this restricted fluid intake his urine output was about 1,000 ml daily, indicating that fluid balance was neutral. The intravenous quinine input over the first four days was 1,000, 0, 500 and 0 mg, respectively. Coma persisted 87 hours and he made an uneventful recovery. The patient's recovery was partly attributed to the moderate doses of both intravenous fluids and quinine. This treatment was successful with cases 7, 8 and 13—16 in particular.

QUININE DOSAGE: Detailed records of quinine therapy were available on four of the six patients who developed pulmonary edema. These men received on average 1,500 mg quinine base intravenously in the first 24 hours (Table 3). The 10 patients who did not develop pulmonary edema received on average 1,055 mg (Table 4). The difference is statistically significant ($t = 5.1$, $p < 0.001$); however, there was no evidence that quinine caused pulmonary edema. For example, Case No. 10 was admitted in deep coma and apparently moribund. He received 1,500 mg quinine intravenously in 1,500 ml daily for the first two days. A toxic concentration of serum quinine was produced (14.1 mg./L) but there was no evidence of pulmonary edema. Case No. 11 also received 1,500 mg of quinine daily intravenously and his cerebral state appeared to deteriorate after each dose of quinine, but intravenous fluids were restricted to 1,500 ml daily and pulmonary edema did not develop. Similarly Case No. 12 was overdosed with quinine since the serum quinine concentration rose to 20.6 mg/L. Pulmonary edema did not develop but the quinine appeared to precipitate aphasia and coma.

DISCUSSION: There are three lines of evidence that pulmonary edema in falciparum malaria is usually caused by therapy and not by the disease.

Firstly, fatal pulmonary edema (or acute pulmonary insufficiency) is rarely mentioned in the older literature but has only recently emerged as a serious and challenging complication of acute falciparum malaria (2). If pulmonary edema were frequently an integral part of the disease then it should appear prominently throughout the literature. However, some textbooks do not mention pulmonary edema (7, 8) and, in another, only one case is mentioned (9). This is in marked contrast to the extensive descriptions of cerebral and other complications. Pulmonary edema has emerged as a clinical problem in the disease coincidentally with the extensive use of intravenous fluids.

Secondly, 80 per cent (21/26) of recent cases of pulmonary edema described in this and other studies (1, 2, 10, 11) developed after admission to hospital. At least one other patient had received intravenous fluids before admission. This suggests that some cases are due to therapy.

Thirdly, in most patients who develop pulmonary edema, there is evidence of a high fluid intake or a positive fluid balance. A positive fluid balance did not occur in our successfully managed patients. It was striking how often the urine output matched the restricted intravenous fluid intake. Either insensible loss is an overrated factor in falciparum malaria or water is produced by tissue catabolism in the disease.

Our adult Thai patients who developed pulmonary edema received an average of 1,800 ml fluid intravenously in the first eight hours after admission and altogether an average of 2,800 ml in the first 24 hours. The patients who did not develop pulmonary edema received an average of 560 ml in the first eight hours and 1,180 ml in the first 24 hours.

Similarly, in Vietnam, pulmonary edema did not develop in any of 73 patients with recrudescent falciparum malaria who received 1,500 ml of intravenous fluid daily (12). These data suggest that re-interpretation of some recent studies is indicated.

In their first paper Punyagupta et al. described two patients. In Case No. 1, the fluid input in the first four days was 17,855 ml and the output was 8,850 ml, giving a positive fluid balance of 9,000 ml, which the authors did not discuss; but during this time the patient developed pulmonary edema. Between the fifth and seventh days the fluid intake was 7,585 ml and output 11,550 ml, giving a negative balance of 3,965 ml. During this time diuretic therapy and many other drugs were given and the pulmonary edema resolved. During the seven days of observation, the average daily fluid intake was 3,634 ml and output of urine was 2,914 ml, which is very high. It should be remembered that homeostasis can be achieved with as little as 500 ml urine daily (14).

In the second patient, pulmonary edema developed on the second hospital day during which the fluid intake was 4,750 ml and output 1,950 ml. Although the diuretic furosemide was administerred to both patients, the authors attributed survival to the treatment with heparin, dexamethasone, dextran and antimalarial drugs. These two patients were included in the total of 12 described in the second report (2). They claimed that fluid overload did not occur, and preferred the term acute pulmonary insufficiency; but they gave no information on fluid balance in their other 10 cases.

Brooks et al. described fatal pulmonary edema in five patients of whom four developed the complication between the third and tenth hospital days (1). In these four patients the average daily fluid intake was 2,900 ml and output 1,800 ml, giving an average positive fluid balance of 1,100 ml daily. Their fifth patient did not receive intravenous fluid before the onset of pulmonary edema and fluid overload was presumably not a factor in that patient.

In three of our patients and probably in many others described in other reports, fluid overload appeared to cause both pulmonary edema and coma (e.g., cerebral edema).

Dehydration is either a rare or non-existent feature of falciparum malaria. Metabolic studies have demonstrated hyponatremia, an increased plasma volume but no abnormality of total body water in many patients with acute falciparum malaria (25, 16). Thus, it is surprising that having demonstrated an increased plasma volume in falciparum malaria, Brooks et al, dismissed fluid overload as being a factor in their patients with pulmonary edema (1).

Miller et al. confirmed the hyponatremia, considered that the cause was salt depletion and water retention and said "serious errors in treament can arise from the blanket assumption that all malaria patients are dehydrated and routinely in need of intravenous water and electrolytes. In those with severe anemia and cardiac disease this could precipitate pulmonary edema" (17). This viewpoint had been expressed before (18). Tigertt (personal communication) has confirmed that many instances of pulmonary edema among U.S. troops with falciparum malaria in Vietnam were due ta fluid overload. In retrospect most patients (U.S. military in Vietnam) with edema in falciparum malaria had a progressive weight gain before the onset of dyspnea in contrast to the usual weight loss of 1.7 to 2.6 kg in the disease (19).

There is evidence, therefore, that in falciparum malaria fluid overload can be produced by intravenous fluid therapy. Extreme thirst is a frequent symptom in the disease, which raises the question whether excessive oral intake of fluids could sometimes precipitate pulmonary edema and coma.

The central venous pressure (CVP) has been normal when measured in falciparum patients with pulmonary edema (1, 2, 11). Our interpretation would be that fluid overload can precipitate pulmonary edema without increasing the CVP. Damage to the pulmonary tissues is probably common in falciparum malaria; thus, pulmonary edema will occur more readily than in healthy lungs.

Since fluid restriction is important in falciparum malaria, the indications for intravenous therapy need to be clearly defined. Inadequate oral intake is obviously an indication. As a minimum, intravenous fluids

are needed to prevent oliguria (urine output less than 400 ml daily) and as a vehicle when intravenous quinine therapy is needed.

Some cases of pulmonary edema or acute pulmonary insufficiency in falciparum malaria are probably due to the disease and are not related to therapy. Deaton reported two patients in whom respiratory symptoms occurred before treatment (11). Fluid balance was negative in one patient and slightly positive in the other.

There is evidence for several pulmonary lesions in falciparum malaria (Table 5). Non-fatal bronchitis or pneumonia is often mentioned in the older literature (20, 21). Clinically diagnosed pulmonary edema, which can resolve with therapy, has been discussed in this paper. Pulmonary edema is a terminal event detected at autopsy in some patients dying of cerebral malaria or other complications of the disease (22).

Most patients with malaria are admitted to remote hospitals. We found in a remote hospital (without facilities for CVP and detailed body weights and urine outputs) that the restriction of fluid intake greatly reduced the incidence of pulmonary edema. This finding is especially applicable to other remote hospitals where fluid intake can be regulated but CVP, body weight and urine output cannot always be determined.

CONCLUSIONS: Pulmonary edema in falciparum malaria may be caused by excessive fluid intake. The present study has shown that the incidence of pulmonary edema can be greatly reduced if fluid input is restricted. One thousand five hundred milliliters of fluid daily (including blood transfusions) or 500 ml every eight hours is the maximum safe intake in full-sized adults. In smaller adults (e.g. many Thais), 1,000 ml is the optimum daily intake. Children should receive proportionately smaller volumes. A positive fluid balance should be avoided if urine output is adequate (e.g., about 1,000 ml urine daily in adults). It is wise to avoid intravenous therapy at night when nursing supervision may be limited.

If pulmonary edema occurs, diuretic therapy is logical: e.g., furosemide by slow intravenous injection. Quinine therapy must also be restricted to prevent toxicity and 1,000 mg daily (two doses) is usually sufficient in adults.

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Table 1. Patients with Falciparum Malaria and Coma. Development of Pulmonary Edema Attributed to Intravenous Overhydration

Patient Number	Age (years)	Maximum Asexual Parasite Count (per cu.mm.)	Volume Infused (ml)			
			0–8 Hrs.	8–16 Hrs.	16–24 Hrs.	Total 0–24 Hrs.
1	20	317800	1450	0	1000	2450
2*	30	94600	2000	0	1000	3000
3	30	639600	1650	350	1000	3000
4	16	96500	1500	0	500	2000
5*	22	777600	1000	500	500	2000
6*	18	103600	3000	1000	500	4500
Average	23	338300	1767	308	750	2825

* Fatal cases

Table 2. Patients with Falciparum Malaria and Coma. Absence of Pulmonary Edema Attributed to Optimal Intravenous Hydration

Patient Number	Age (years)	Maximum Asexual Parasite Count (per cu.mm.)	Volume Infused (ml)			
			0–8 Hrs.	8–16 Hrs.	16–24 Hrs.	Total 0–24 Hrs.
7	35	719800	1000	0	1000	2000
8	16	6300	900	100	0	1000
9	30	307600	630	370	500	1500
10*	45	131200	500	500	500	1500
11	24	772600	500	500	500	1500
12	26	10800	500	0	500	1000
13	42	836200	500	0	500	1000
14	14	12800	350	460	0	810
15	53	2000	500	0	250	750
16	14	72300	250	0	500	750
Average	30	287200	563**	193	425	1181**

* Fatal case

** Significantly less ($p < 0.01$) than the input in the patients who developed pulmonary edema (See Table 1).

Table 3. Patients with Falciparum Malaria, Coma and Pulmonary Edema Intravenous Quinine Dosage First 24 Hours

Patient Number	Quinine Infused (mg)				Comment
	0–8 Hrs.	8–16 Hrs.	16–24 Hrs.	Total 0–24 Hrs.	
1	1000	0	500	1500	
2					Mostly chloroquine therapy
3	850	150	500	1500	
4	1000	0	500	1500	
5	1000	0	500	1500	
6	?	?	(500)	?	Treatment before admission
Average	963	37	500	1500	

Table 4. Patients with Falciparum Malaria and Coma but without Pulmonary Edema
Quinine Dosage First 24 Hours

Patient Number	Quinine Infused (mg)				Comment
	0–8 Hrs.	8–16 Hrs.	16–24 Hrs.	Total 0–24 Hrs.	
7	500	0	500	1000*	
8	950	50	0	1000*	
9	650	350	500	1500	
10	500	0	750	1250	Died
11	500	500	500	1500	Quinine toxicity
12	500	0	500	100.*	
13	500	0	500	1000	Quinine toxicity
14	350	450	0	800*	
15	500	0	250	750*	
16	500	0	250	750*	
Average	545	135	375	1055	

* Modest amounts of quinine and intravenous fluids were associated with an optimal clinical response in these patients.

Table 5. Pulmonary Lesions in Falciparum Malaria

Lesion	Causation	Comment
1. Pneumonitis	Disease	Non–fatal
2. Bronchitis	Disease	Non–fatal
3. Pulmonary edema	Therapy	Fatal or reversible
4. Pulmonary edema or "acute pulmonary insufficiency"	Disease	Fatal (diagnosed before death)
5. Pulmonary edema	Disease	Autopsy finding

Jaundice in Falciparum Malaria

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OBJECTIVE: a) To determine whether a correlation exists between the total serum bilirubin and the parasite count, and b) to determine if serum bilirubin correlates with certain other parameters in *falciparum* malaria.

DESCRIPTION: Published reports of the prevalence of jaundice in falciparum malaria have varied from one per cent to 72 per cent in a group of 46 fatal cases in Africa. Jaundice was a common feature in therapeutic malaria; e.g., in one series of 400 syphilitics inoculated with malaria, six per cent developed jaundice (1). Apart from jaundice, abnormality of hepatic function in falciparum malaria is common (2). Hepatomegaly can be demonstrated during an acute attack in approximately half the cases of malaria (3); following recovery, the liver returns to its normal size within a few days.

DESCRIPTION: This study was conducted at the Trad Provincial Hospital in Southeast Thailand. Between 11 January 1973 and 21 July 1974 a malaria clinic was operated daily from 8 AM to 9 AM. Patients were self–referred or referred by the hospital doctors. Of 11,241 patients who were screened 4,824 had falciparum malaria and 929 had vivax malaria. The present survey was conducted on all adult (over age 15) patients with malaria seen in the clinic during a 40 day interval. For each patient with malaria, venous blood was used to determine parasite density (except for those with vivax malaria) by the method of Earle and Perez, and the packed cell volume (hematocrit) using a microcapillary centrifuge. On each serum the bilirubin, creatinine, SGPT, and alkaline phosphatase concentrations were measured using an Auto–Analyzer.

PROGRESS: Slight increases in total serum bilirubin (to between 1–2 mg per cent) occurred in 55 per cent of the patients with falciparum and 42 per cent of the patients with vivax malaria (Table 1). However, if jaundice is defined as a total serum bilirubin over 2.0 mg per cent, then it did not occur in any of the 24 patients with vivax malaria. Clinical jaundice is rarely reported in vivax malaria. Of the 236 patients with falciparum malaria and an average parasite count of 26,000 per cu.mm., 19 per cent had jaundice. Depth of jaundice (as defined above) correlated ($r = 0.35$, $p < 0.01$) with the parasite count (Table 2), as it occurred in only 5 per cent of the patients with a parasite density below 1,000 per cu.mm. but in 45 per cent of those with a count over 100,000 (Table 1).

The serum bilirubin correlated positively with serum alkaline phosphatase, SGPT, serum creatinine, parasite density and negatively with the packed cell volume. The serum alkaline phosphatase correlated with SGPT, serum bilirubin and parasite density. The SGPT only correlated with serum alkaline phosphatase and serum bilirubin.

In most patients recovery from jaundice was rapid and coincided with successful treatment with quinine of the falciparum malaria. For example, in one patient the total serum bilirubin was 8.0 mg per cent on admission but had decreased to 0.9 mg per cent five days later. The severity of malaria tended to be less in the older patients as shown by the negative correlation between age and parasite count ($r = -0.19$, $p < 0.05$).

There was an inverse correlation between the total serum bilirubin and the hematocrit ($r = -0.295$, $p < 0.01$); however, not all of the jaundiced patients were anemic. Anemia was more common in the seriously ill patients; as the hematocrit correlated inversely with the parasite count ($r = 0.322$, $p < 0.001$).

DISCUSSION: There have been few studies using the direct (and accurate) method for counting malaria parasites in patients with naturally acquired falciparum malaria. Thus, it is not surprising that this is the first study to show a correlation between the parasite count and the total serum bilirubin. This supports the strong clinical impression that jaundice is more common in patients seriously ill with falciparum malaria.

We found a correlation between anemia and jaundice which confirms that hemolysis is one factor in the etiology of jaundice in malaria. The serum bilirubin also correlated with the other indices of hepatic function (SGPT and alkaline phosphatase) which suggests that the jaundice is at least partly due to liver damage. Thus, the production of excess bilirubin is the result of excessive hemolysis but the failure of its removal from the blood may be due to liver damage (4). Besides the SGPT and the alkaline phosphatase, the serum bilirubin also correlated with the serum creatinine, the parasite count, and the packed cell volume. Thus, the serum bilirubin seems to be the most useful index of hepatic function in falciparum malaria, and is also a useful guideline to estimate the severity of the disease.

Table 1. Total Serum Bilirubin (mg per cent) vs Parasite Count in 236 Patients with Falciparum Malaria*

Falciparum Range of Parasite Count (per cu. mm.)	Patients with Bilirubin >1.0 mg%	Patients with Bilirubin >2.0 mg%	Mean Bilirubin (± SEM)**	Range
0–99	24% (4/17)	6% (1/17)	0.90 ± 0.14	0.4–2.9
100–999	38% (8/21)	5% (1/21)	0.93 ± 0.10	0.3–2.1
1000–9999	47% (31/66)	17% (11/66)	1.27 ± 0.10	0.4–4.9
10000–99999	59% (60/101)	17% (17/101)	1.61 ± 0.17	0.5–12.0
100000+	87% (27/31)	45% (14/31)	3.56 ± 0.84	0.4–20.6
Average 26000	55% (130/236)	19% (44/236)		
Patients with Vivax Malaria	42% (10/24)	0% (0/24)	0.99 ± 0.06	0.4–1.7

* Includes data on 24 patients with vivax malaria

** Standard error of the mean

Ross (5) reported that quinine therapy sometimes induced an increase in the serum bilirubin level. But we found that reduction of the parasite density by quinine is usually associated with a rapid reduction in the serum bilirubin concentration.

We did not find jaundice in our 24 patients with vivax malaria (nor in others that we have studied), although jaundice in vivax malaria has been reported by some workers. It is probably rare in pure infections with *P. vivax;* this indicates the importance of accurate species identification.

SUMMARY: Jaundice (defined as a total serum bilirubin over 2 mg per cent) occurred in 19 per cent (44/236) of a group of patients with falciparum malaria and an average parasite density of 26,000

asexual parasites per cu.mm. The serum bilirubin correlated positively with the parasite density ($p<0.01$) and inversely with the packed cell volume ($p<0.01$); this confirms that the jaundice is at least partly due to hemolysis. The serum bilirubin also correlated positively with the SGPT and the serum alkaline phosphatase levels, which indicates that hepatic damage also contributes to the jaundice. The prevalence of jaundice varied from 6 per cent in patients with a parasite count less than 1,000 per cu.mm. to 45 per cent in patients with a parasite count over 100,000. Jaundice was not present in any of 24 patients with vivax malaria.

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Table 2. Intercorrelations of Seven Parameters in 236 Patients with Falciparum Malaria*

Parameters	Age	Parasite Count	Hematocrit	Bilirubin	SGPT	Alk Phos	Creatinine
Age	1.0	−0.19	0.157	0.008	−0.044	−0.032	0.03
Parasite Count		1.0	−0.322	0.352	0.151	0.230	0.205
Hematocrit			1.0	−0.295	−0.007	−0.169	−0.158
Bilirubin				1.0	0.351	0.274	0.226
SGPT						0.340	0.094
Alk Phos						1.0	0.052
Creatinine							1.0

* With $n=236$ the following values of r are associated with the listed probability levels:

r	p
> 0.296	<0.001
> 0.225	<0.01
> 0.18	<0.05

Anemia in Malaria and the Role of Blood Transfusion

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OBJECTIVE: To determine the prevalence of anemia in falciparum and vivax malaria. To determine the rate of recovery from anemia in both diseases. To determine the role of blood transfusion (if any) in the management of the anemia.

BACKGROUND: There is little information on the prevalence of anemia in malaria. Anemia is a well-known complication of falciparum malaria but is less commonly a problem in vivax malaria. Comparative studies on the rate of recovery from anemia in falciparum and vivax malaria are not available. Most standard sources recommend blood transfusions as part of the general therapy for the hemolytic problem (2, 3). References, however, are extremely vague, and give no data on the results of blood transfusion.

DESCRIPTION: Anemia in this study is defined as a hematocrit (packed cell volume) below 35 per cent. Hematocrits have been determined on several hundred patients with falciparum malaria over the last few years. The hematocrit and other parameters were correlated in 236 unselected patients with falciparum malaria. Some data is also available on a small group of patients with vivax malaria. Daily hematocrits in hospital and weekly hematocrits during follow-up have been taken on the SEATO ward at Prachinburi Hospital since February 1975.

PROGRESS: In an unselected series of 142 patients with falciparum malaria, 63 (44 per cent) developed a hematocrit below 35 per cent during their hospital course and could therefore be considered to have developed anemia. Data on a much larger group of patients is being analyzed.

In a study of jaundice in falciparum malaria described elsewhere, two relevant correlations were discovered. The hematocrit correlated negatively with the parasite count ($r = -0.32$, $p < 0.001$); in other words, anemia is more common in patients with higher parasite counts. Also, the hematocrit correlated negatively with the total serum bilirubin ($r = -0.29$, $p < 0.01$) which indicates that anemia is more common in falciparum patients who are jaundiced.

Clinically the anemia of falciparum malaria improves steadily once the infection has been brought under control. The data in Figure 1 support this impression. Depicted are serial hematocrits on three patients who developed a worsening anemia after admission. When the malaria was successfully treated with quinine, the hematocrit steadily increased without blood transfusion or hematinics. These results are typical of most falciparum patients with anemia that we have studied.

Altogether five patients with severe anemia (defined as a hematocrit below 15 per cent) have been successfully managed without blood transfusion. One interesting patient is shown in Figure 2. This five year old boy was admitted almost in coma and with a parasite count of 41,000 per cu.mm. The patient weighed 15 Kg. He received six doses of intravenous quinine at the rate of two infusions daily administered in a total of 500 ml fluid daily. The parasitemia cleared in 64 hours but the patient remained in coma for five days. On day 6 the patient was conscious but very weak. The hematocrit had decreased from 19 to 12 per cent. A 300 ml blood transfusion was given and the hematocrit increased to 19 per cent. On the following day a second transfusion of 200 ml was given and the hematocrit increased further to 23 per cent. A dose of pyrimethamine with sulfadoxine (one tablet) was given and the patient made a steady recovery.

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1 Trad Provincial Hospital

DISCUSSION: Our preliminary results indicate that about 45 per cent of Thai patients with falciparum malaria develop anemia (defined as a lowest recorded hematocrit below 35 per cent). In one small study of U.S. troops with falciparum malaria in Vietnam, 22 per cent had anemia by this criterion. In another group of 50 U.S. troops, 58 per cent had a hematocrit less than 38 per cent.

Hemolysis is the principal cause of anemia in falciparum malaria but impairment of red cell production may also occur. The question arises as to what extent the anemia in falciparum malaria is due to background malnutrition or iron deficiency rather than to the disease itself. Our studies suggest that this is a minor factor. Anemia has not been a problem in the convalescence of our patients. The data in Figure 1 is typical in that it shows an increase in the hematocrit once the maluria is brought under control. Slightly low levels of the hematocrit (around 35 per cent) do persist in some patients in convalescence. We are now studying the effect of iron therapy in patients whose hematocrits have stabilized in convalescence. The probable answer is that anemia in falciparum malaria is mainly due to the disease but in some patients malnutrition, iron deficiency or helminthiasis are minor factors.

The role of blood transfusion (if any) in falciparum anemia needs to be clearly defined. Blood transfusion is recommended by some authorities (2), and indeed Manson (3) implies that transfusion should be considered if the hematocrit falls below 30 per cent. We agree with Canfield (1) who asserts that blood transfusion is rarely required. In patients that we have observed, blood transfusion often results in pulmonary edema. Another sequel of transfusion is severe hemoglobinuria which obviously is due to hemolysis of the transfused cells. In this situation the transfusion has obviously been unsuccessful since the hematocrit does not rise. There are several problems with transfusing blood in hospitals in remote malarious areas. The donor blood may contain malaria parasites. Also it may be difficult to cross-match the blood as precisely as in more sophisticated hospitals in non-endemic areas. It is probably true that red cells transfused during the acute phase of the disease are more likely to be hemolyzed than during convalescence. This concept is supported by the patient depicted in Figure 2; after the parasitemia had been eliminated by quinine; blood transfusion caused a satisfactory increase in the hematocrit. Our largely anecdotal evidence suggests that blood transfusion is rarely indicated in falciparum malaria. A protocol of a controlled study of blood transfusion in falciparum anemia has been submitted to the Ministry of Health of Thailand. Patients who develop a hematocrit between 12 and 20 per cent will be randomly assigned to blood transfusion or conservative management. All patients will receive quinine therapy by intermittent intravenous infusions. Patients with hematocrits below 12 per cent will be treated according to the clinical picture and patients with a hematocrit above 20 per cent will usually be treated conservatively. The patients will be closely followed clinically and detailed relevant hematologic and biochemical tests will be performed.

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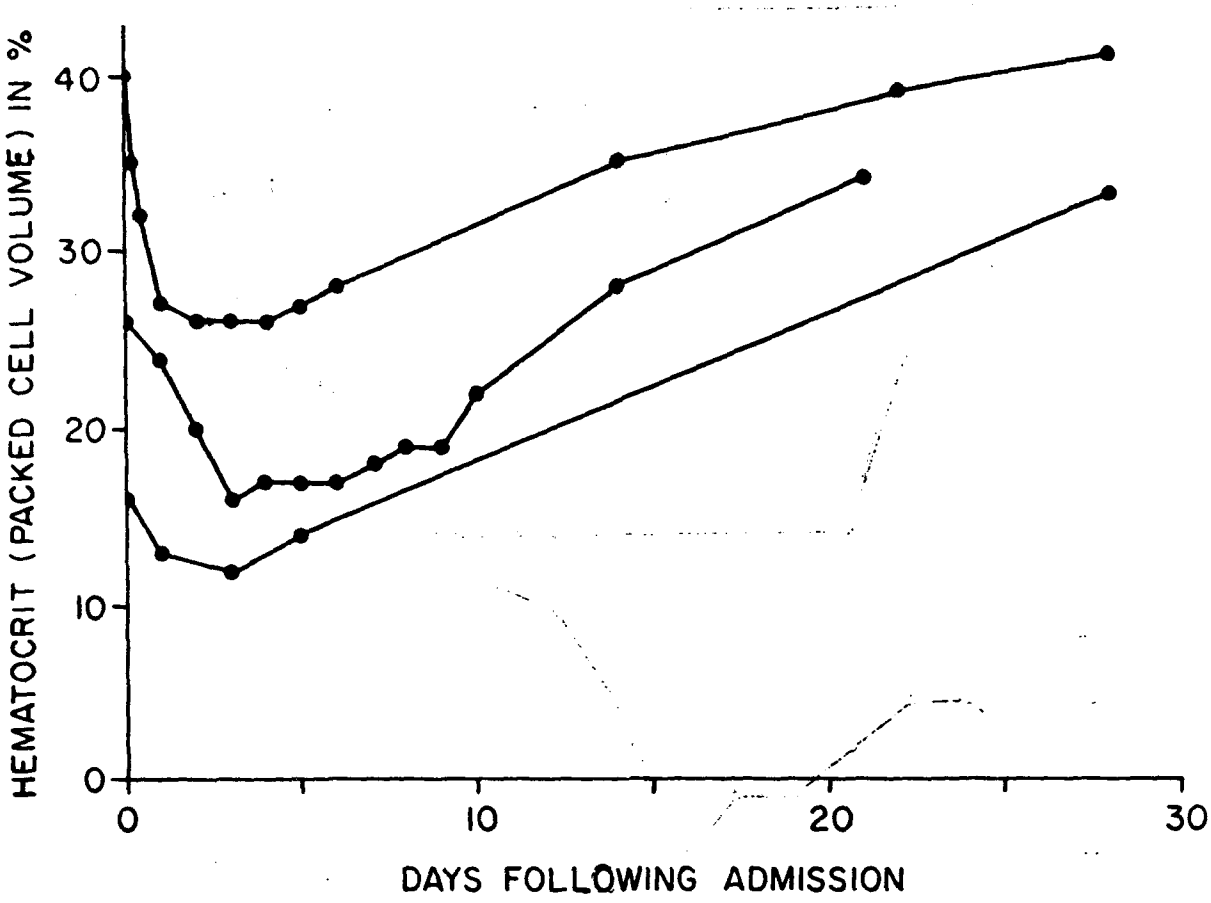

Figure I. Three patients with falciparum malaria. Recovery from anemia was associated with cure of malaria. Blood transfusion and hematinics were not used.

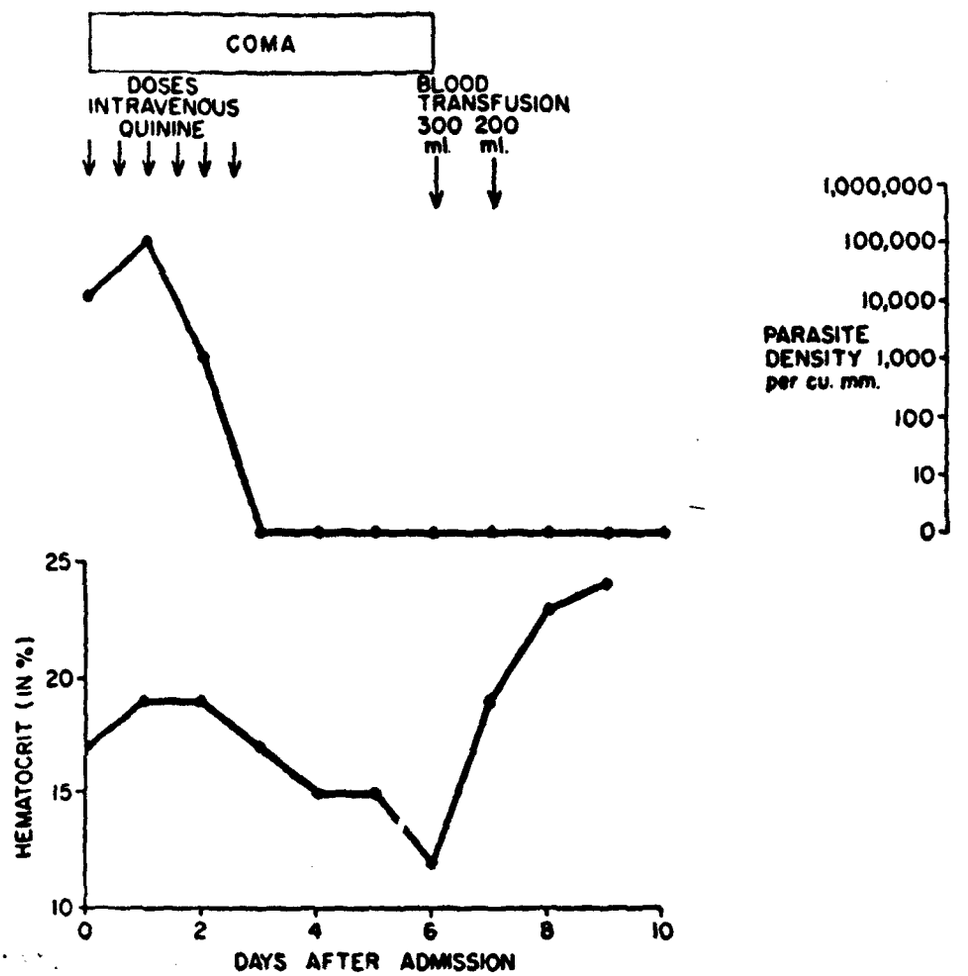

Figure 2 Boy with falciparum malaria aged 5 years. After eradication of parasitemia with quinine therapy, a satisfactory increase in hematocrit followed blood transfusion

Quinine Dosage and Serum Levels in Falciparum Malaria

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BACKGROUND: The World Health Organization has emphasized the need for studying the pharmacology of antimalarial drugs in patients with falciparum malaria (1). Quinine is the only rapidly acting drug for chloroquine-resistant falciparum malaria. In healthy volunteers, quinine by intravenous infusion produced higher plasma quinine levels than did oral quinine (2).

We compared the serum concentrations of quinine following a single dose of one of the three modes of administration, (intravenous infusion, intramuscular injection and tablets) in patients with falciparum malaria, since no information was available on this subject. Serum quinine levels were also determined following repeated administration of the drug at 8 or 12 hour intervals, to determine the optimum dosage interval.

DESCRIPTION: Patients at Trad Provincial Hospital with falciparum malaria comprised the study group. Trad is in a remote area of Thailand 400 km from Bangkok. Quantitative parasite counts on thick blood films were made at least once daily (3).

Serum quinine levels were determined in 73 people following a single dose of the drug; sera were collected before dosing and usually 1, 2, 4 and 8 hours after the oral or intramuscular dose or after the beginning of an infusion. Twelve and 24 hour specimens were collected in a few patients. Thirteen patients were excluded from analysis because quinine was detected in the serum on admission. Sera could not be collected after 10 PM, so complete 1-8 hour data were only available on 35 patients. For technical reasons, urine could not be collected. The patients were randomly assigned to one of five dose regimens: two intravenous (two or four hour infusion), two oral (coated or plain tablets) and one intramuscular. The patients in each group were comparable with respect to age (overall mean 23.7 years) and weight (overall mean 51.8 kg).

Intravenous infusions (IVQ) consisted of 490 mg base (2 ml) of quinine dihydrochloride (The Vitarine Company, New York) injected into an infusion bottle containing 500 ml normal saline. The bottles were infused over a two or four hour interval. All were calibrated and the rate of flow was checked frequently to maintain a constant infusion rate of 125 ml or 250 ml each hour.

The enteric coated tablets of quinine sulfate (Strong Cobb Arner, Cleveland) contained 270 mg quinine base. The usual dose was 540 mg. The plain uncoated tablets (Government Pharmaceutical Organization, Bangkok) contained 250 mg base. The usual dose was 500 mg.

Intramuscular quinine (IMQ) was administered as quinine dihydrochloride (same preparation as used for intravenous therapy) in a dose of 490 mg quinine base (2 ml). The injections were made deeply in the upper and outer quadrant of the buttock.

Serum quinine levels following multiple dosing of the drug were determined in 31 patients (18 of whom had been studied following the initial dose). Nineteen patients received quinine at full dosage every 8 hours and 10 patients every 12 hours. Two patients received half-dosage quinine every 8 hours. With very few exceptions, all doses following the initial dose were oral. In multiple dose studies sera were collected at the time of dosing and at other times (e.g. 1, 2, 4, 8 and 12 hours after a dose). Serum quinine concentrations were determined by the benzene extraction method (4). Measurements were performed using an Aminco-Bowman spectrophotofluorometer (350 mμ. activation; 450 mμ. fluorescence). The parenteral and oral formulations of quinine were assayed and found to contain the designated amount of quinine base ($\pm$ 2 percent).

The half–life (T/2) of quinine was determined by plotting the serum concentrations of quinine against time on semi–logarithmic paper. Data from the single–dose studies were compared using analysis of variance. Following the overall analysis of variance, individual F tests were conducted to determine the location of mean differences (5, 6).

Single Dose: Complete data (serum levels at 1, 2, 4 and 8 hours after the dose) were available on 35 patients. There were seven patients in each of the five treatment groups. The mean serum quinine concentrations for each group are shown in Table 1. The analysis of variance indicated significant differences between the modes of administration and between the hours of sampling (Tables 2 and 3). One hour after initiation of the dose, the two hour infusion produced a significantly greater serum quinine concentration than did intramuscular quinine ($p<0.01$) which produced a significantly greater level than plain tablets ($p<0.01$). Four hours after initiation of the dose the four hour infusion produced a greater serum level than coated tablets ($p<0.05$). At eight hours, coated tablets produced a higher level than the intramuscular injection ($p<0.05$). The serum quinine concentrations following intravenous or oral administration were not significantly different at eight hours (Figure 1).

Serum quinine levels were not available at 12 hours for all 35 patients: the data were pooled (Figure 1). It is noteworthy that the serum quinine concentration 12 hours after dosing was about 6 mg per L following either intravenous or oral therapy.

Details on each of the regimens are now given.

Two Hour Infusion: Initially 10 men received a two hour infusion. Quinine was detected by later testing in the admission serum of three of these men, two of whom developed severe cinchonism (quinine toxicity) at the end of the infusion. Seven men had no quinine in the serum on admission and the average results on these patients were used in the analysis (Table 1). One and two hours after the beginning of the infusion, greater serum quinine levels occurred than with any other regimen. Thereafter the results were similar to those obtained with the four hour infusion. In one patient the serum quinine peaked at 10.8 mg per L at two hours and decreased slowly to 5.7 mg per L at 22 hours. This data suggests a half–life (T/2) of 21 hours. The clinical response (e.g. fever, parasitemia) to the two hour infusion was excellent; however, cinchonism was more common than with any other regimen.

Four Hour Infusion: Greater serum quinine concentrations were achieved with the four hour infusion than with oral or intramuscular quinine. In most patients the clinical response was excellent.

One patient, who was jaundiced, became aphasic during the infusion and was struggling and comatose at the end, at which time the serum quinine level was 8.8 mg per L. Sixteen hours later he could speak and since no further therapy had been given, a second quinine infusion was begun. During the second infusion he again became aphasic. The patient then received a single dose of pyrimethamine with sulfadoxine (Fansidar) and made a steady recovery. Neurotoxic hypersensitivity to quinine was diagnosed. In the other patients, the four hour infusion caused minimal side–effects.

Coated Tablets: The mean serum concentration of quinine one hour after the dose was only 0.8 mg per L. A peak serum quinine level of 7.6 mg per L occurred at four hours.

The clinical response was unsatisfactory in one patient; four hours after dosing his serum quinine was 6.1 mg per L. He complained of weakness, dizziness, cough and abdominal pain. Three episodes of diarrhea had occurred since admission. The patient improved after 250 mg quinine was infused, although further serum quinine levels were not obtained. One patient developed urticaria on the face and chest four hours after dosing that persisted for several hours and was attributed to the quinine. Mild cinchonism occurred in several patients.

Plain Tablets: The mean serum quinine levels after plain tablets were higher than after coated tablets at one and two hours but lower at four and eight hours. With seven patients in each group the one hour

data were not significantly different (Table 1). One hour data was available on several other patients not included in the group of 35, because not all 2–8 hour specimens had been obtained. In a total of 12 patients who received coated tablets, the mean one hour level was 0.7 mg per L. In 12 patients who received plain tablets, the mean level was 2.7 mg per L. The difference is significant ($t = 2.5$, $p < 0.05$).

In one patient the clinical response was unsatisfactory. Coma supervened two hours after therapy when the serum quinine level was only 0.4 mg per L. Thereafter the absorption of quinine was much improved (serum quinine level at four hours was 10.6 mg per L). The plain tablets often caused mild cinchonism but no serious toxicity was encountered.

Intramuscular Injection (IMQ): The serum concentration–time curve following intramuscular quinine was flat and did not rise appreciably above 6 mg per L.

One patient had a poor clinical response. Following the injection at 1100 hours, his parasite count increased from 12,000 to 217,000 per cu.mm. at 1700 hours. His serum quinine concentration was then only 3.1 mg per L. Intravenous quinine was then infused with a satisfactory response.

Multiple Dose Studies: Eighty percent (16/19) of the patients who received quinine at eight hour intervals developed some degree of cinchonism and in most patients this was associated with serum quinine concentrations assumed to be in the toxic range (over 10 mg per L). Only 30 percent (3/10) of the patients on 12 hour dosing developed cinchonism. The difference is significant (Chi square $= 6.9$, $p < 0.01$).

An example of overdosage with quinine given every 8 hours is shown in Figure 2. Early in therapy a high fever was associated with a raised serum bilirubin and a very high concentration of serum quinine (17.1 mg per L). Improvement was associated with a decrease in fever and bilirubin.

An example of an optimum response to quinine given every 12 hours is depicted in Figure 3. Clinical improvement was associated with a decrease in serum bilirubin and in serum quinine despite constant therapy. The approximate half–life (T/2) decreased from 21 to 14 to 12 hours during therapy.

On the other hand, in two other patients, therapeutic concentrations of serum quinine were maintained with a half–dose (one tablet) given every 8 hours.

The average half–life of quinine in falciparum malaria, derived from all available data on 17 patients was 18.8 hours (range 12–31).

DISCUSSION: The serum concentrations of quinine following oral therapy were low in relation to those following intravenous therapy for the first four hours after dosing. Thereafter the serum levels were the same. These data confirm the clinical impression that the intravenous route is more effective than the oral route for at least the first dose of quinine in falciparum malaria. Plain tablets produced significantly greater serum concentrations than coated tablets one hour after dosing. If the first dose has to be oral then plain are preferable to coated tablets. There was no evidence that cinchonism was less common in the patients who received coated rather than plain tablets.

Intramuscular quinine produced a significantly higher one hour serum quinine concentration than did oral quinine, and might occasionally be preferable to oral quinine for the first dose in situations where intravenous infusions are not available. However we have seen several patients in whom IMQ was clearly less effective than IVQ. The average serum quinine level following IMQ barely rose above 6 mg per L in distinction to the other modes of administration. The conclusions are that, in this study, 6 to 10 mg per L was the usual therapeutic range for quinine and that IMQ did not always produce sufficiently high levels. The bioavailability of quinine was least by the intramuscular route, a situation analogous to that for digoxin (7) and diphenylhydantoin (8). One dose of IMQ may be satisfactory in mild cases of falciparum malaria but repeated dosing is not indicated because gluteal abscesses may be produced (9).

intravenous infusions, either of two or four hours duration, produced serum quinine concentrations above 6 mg per L from the first hour onwards. The two hour infusions naturally produced higher levels but also more frequently caused toxicity. A two hour infusion can be considered for the initial dose but not for maintenance therapy. In two patients with quinine in the serum on admission, the two hour infusion produced very high serum quinine levels with toxicity. We have given IVQ with infusion times from 1 to 16 hours (10, 11). Lengthy infusions can cause arm discomfort and be inconvenient for the patient. The four hour infusion is the optimum form for quinine administration since therapeutic but not toxic levels of serum quinine are usually produced.

In this study the average half-life (T/2) of quinine was 19 hours in falciparum malaria which compares with the T/2 of 10 hours in healthy volunteers that can be deduced from another study (2). The T/2 of quinine is greater in volunteers with falciparum malaria than in the same volunteers without malaria (12). Thus in falciparum malaria the metabolism of quinine may be impaired presumbly due to liver dysfunction since quinine is mainly metabolized in that organ (13). Conversely quinine (2) or, its optical isomer, quinidine (14), can cause liver damage. A vicious cycle may occur: if a patient with falciparum malaria has hepatitis, the half-life of quinine is prolonged; thus routine doses of quinine may result in toxic levels of serum quinine; the latter may result in greater damage to the liver; the metabolism of quinine is thus further impaired; consequently even greater levels of serum quinine result.

Therefore, in most patients with falciparum malaria, lower or less frequent doses of quinine should produce the same serum quinine as higher doses in healthy patients. Our data confirm this theory. In many patients, maintenance therapy with full doses of quinine every 8 hours produced toxicity and high serum quinine levels, whereas quinine every 12 hours produced a satisfactory clinical response and therapeutic levels. This is not surprising since the average serum quinine level was still about 6 mg per L 12 hours after the initial dose of oral or intravenous quinine. On the other hand, therapeutic concentrations of serum quinine can be maintained with a half-dose (one tablet) given every 8 hours. Dosing every 12 hours might produce greater fluctuations of serum quinine concentration than will dosing every 8 hours; however, it is not known whether fluctuation or constancy in the serum quinine level is more effective. In tropical practice, drugs are usually given three times a day (e.g. 0800, 1200 and 1700 hours) rather than every 8 hours, so administration every 12 hours is easier to accomplish.

In several patients, high serum quinine levels occurred early in therapy, but despite constant maintenance therapy, a sharp decrease occurred associated with improvement in clinical state, fever and hepatic function. Other studies have found similar results (15, 16). We attribute the decrease in serum quinine levels to improved metabolism of quinine consequent upon improvement in hepatic function.

Reduced doses of quinine (e.g. 600 mg base intravenously daily) must be administered in patients with falciparum malaria complicated by renal failure (17, 18). In those studies the impairment of quinine metabolism may have been partly due to concomitant hepatic damage.

Aphasia and coma were caused by quinine in one of our patients at a time when the serum quinine was only 8.4 mg per L. Coma in falciparum malaria is sometimes caused by quinine rather than by the disease (i.e. quinine coma). However "It is sometimes difficult to distinguish between true quinine toxicity and complications of the diseases for which the drug is given" (13).

The WHO recommends 650 mg (540 mg base) three times daily as the standard dose of quinine (i.e. six tablets daily) (1). However the labels on the bottles of tablets used in this study recommend three tablets daily for two days followed by two tablets daily for five days. The difference between six tablets and 2–3 tablets daily is considerable. The paradox can be explained by the fact that the WHO recommendation in based upon several studies with Americans in Vietnam whereas the manufacturer's recommendations probably take into consideration the lower average body weight of people indigenous to the malarious areas. U.S. soldiers weighing 75 kg usually tolerate about 1500 mg of quinine base per day (20 mg/kg) but the same dose in 50 kg Thais is 30 mg/kg.

Our studies indicate that 20 mg/kg/day is sufficient in the average patient with falciparum malaria. This is the dose recommended by Fletcher (9). One infusion of 10 mg/kg/day is probably the optimum dose for falciparum patients with severe renal, hepatic or cerebral complications. Thus the more seriously ill the patient, the lower should be the dose of quinine.

It is of interest that 15 percent of the patients (13/86) in the single dose study had detectable quinine in the serum on admission. This is an indication of the prevalence of therapy before admission. Quinine toxicity may be compounded if this possibility is not considered.

SUMMARY: Patients with chloroquine–resistant falciparum malaria were studied. In 35 patients serum quinine concentrations were determined 1, 2, 4 and 8 hours after the initial dose of quinine or after the beginning of an infusion. The patients were randomly assigned to one of five dose regimens: two hour intravenous infusion, four hour intravenous infusion, coated tablets, plain tablets and intramuscular injection.

Intravenous quinine was better absorbed than oral quinine from one to four hours but the serum levels were not significantly different at eight hours. A four hour infusion is the optimum method of administering the first dose of quinine. Intramuscular quinine produced a significantly greater serum quinine level than oral quinine at one hour but a significantly lower level at eight hours. Further patients were studied and it was shown that plain tablets produced a significantly greater mean serum quinine concentration than coated tablets one hour after dosing.

In Thai patients (average weight 50 kg), full dose quinine (540 mg base) every 8 hours often produced toxic serum concentrations, whereas dosing every 12 hours was optimal therapy but still produced cinchonism in 30% of patients.

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Table 1. Serum Quinine Concentrations (mg/L) Following Intravenous Infusion, Oral or Intramuscular Administration

Hour	Intravenous 490 mg/2 Hrs		Intravenous 490 mg/4 Hrs		Oral 540 mg/Coated		Oral 500 mg/Plain		Intramuscular 490 mg	
	No.	Mean ± SEM	No.	Mean ± SEM	No.	Mean ± SEM	No.	Mean ± SEM	No.	Mean ± SEM
0	7	0	7	0	7	0	7	0	7	0
1	7	8.7 ± 1.1	7	6.0 ± 0.6	7	0.8 ± 0.4	7	2.6 ± 0.9	7	5.5 ± 0.9
2	7	10.7 ± 1.2	7	7.7 ± 0.6	7	5.1 ± 1.4	7	5.3 ± 0.8	7	6.1 ± 0.9
4	7	9.4 ± 1.0	7	9.8 ± 0.9	7	7.6 ± 1.1	7	7.0 ± 0.6	7	6.1 ± 0.9
8	7	8.3 ± 0.9	7	7.6 ± 0.9	7	7.3 ± 1.0	7	6.8 ± 0.8	7	5.3 ± 0.8
12	6	7.2 ± 1.0	5	5.2 ± 0.7	2	7.2 ± 1.9	2	3.7 ± 2.1	2	3.4 ± 1.1

Table 2. Analysis of Variance (Hours 0 to 8)

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Square	F	p
Hours	199.5	3	66.5	23.3	<0.001
Error	68.4	24	2.85		
Mode of Administration	351.9	4	87.9	13.3	<0.001
Hours x Mode	171.1	12	14.3	2.2	Not Significant
Error	634.9	96	6.6		

Table 3. Statistical Significance of Mean Values of Clinical Importance

Mode*	Hour**	F	p
IVQ 2 v IMQ	1	5.4	< 0.01
IMQ v Oral (Plain)	1	4.5	< 0.01
Oral (Plain) v Oral (Coated)	1	1.7	Not Significant
IVQ 4 v Oral (Coated)	4	2.6	< 0.05
IVQ 2 v Oral (Plain)	8	1.2	Not Significant
Oral (Coated) v IMQ	8	2.1	< 0.05

* IVQ 2 = Quinine infused in 2 hours
IVQ 4 = Quinine infused in 4 hours
IMQ = Intramuscular quinine
** Hour refers to number of hours elapsed since treatment begun

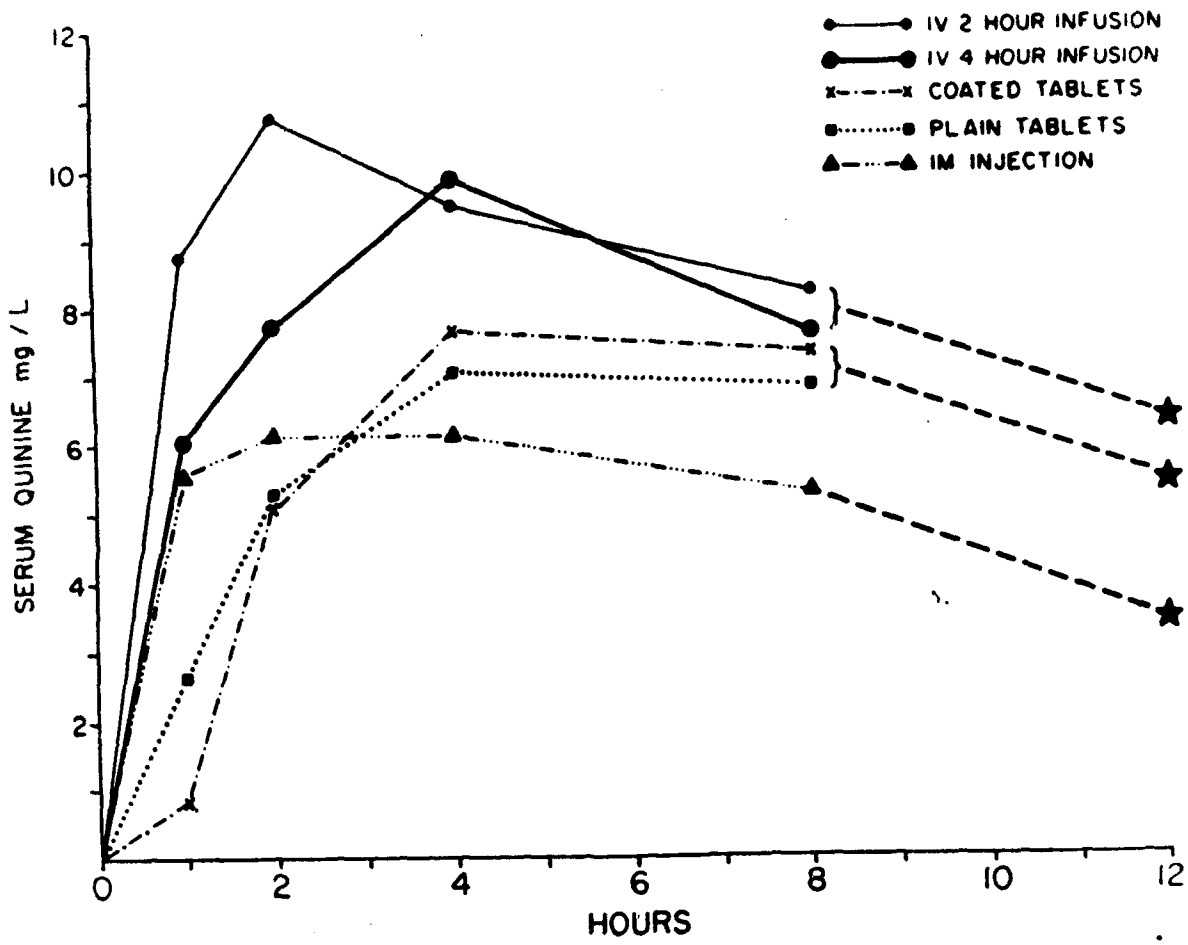

Figure 1. Mean serum quinine concentrations following a single dose of quinine using 5 different modes of administration. There were 7 patients in each group. Complete data are not available at 12 hours.

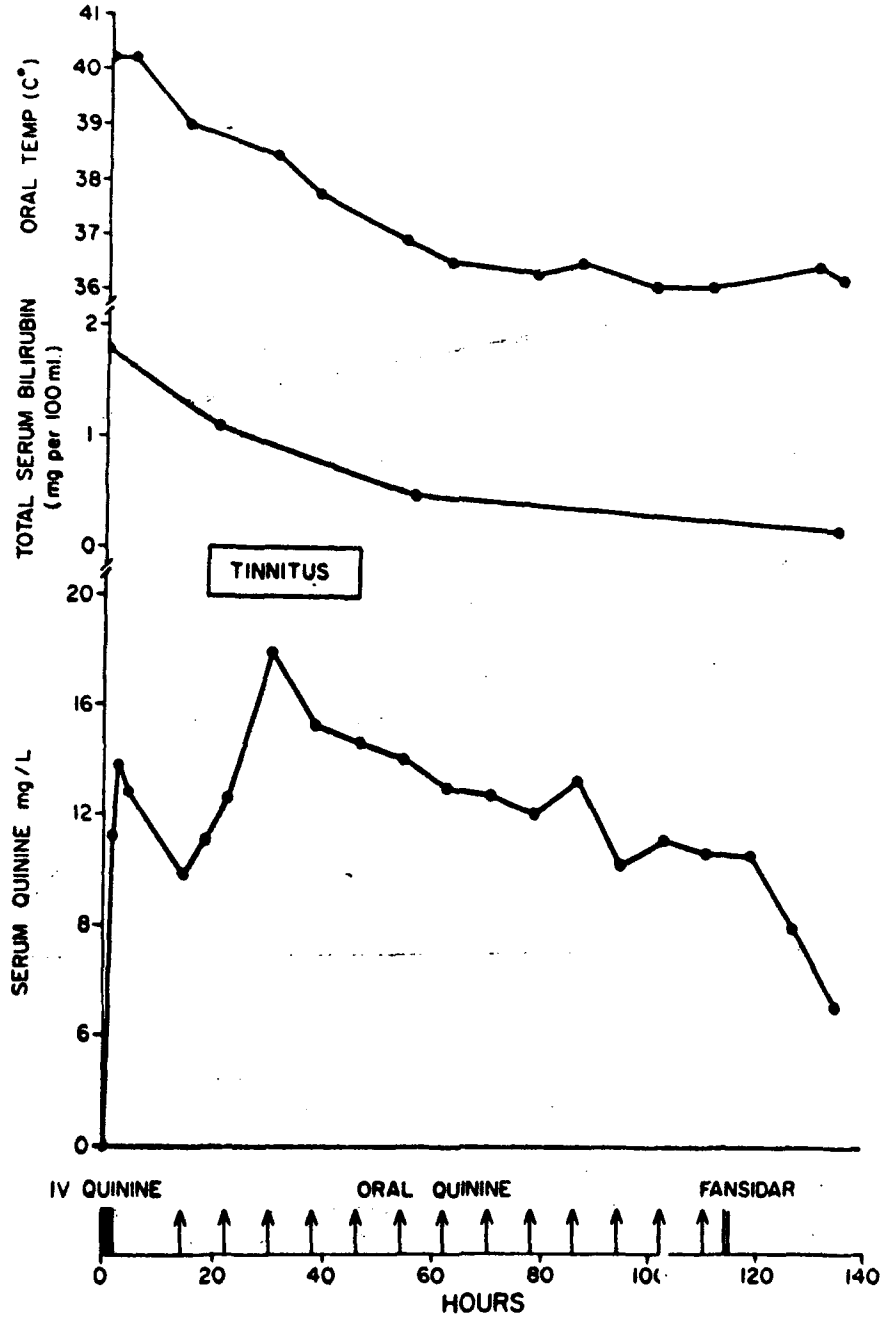

Figure 2. Routine quinine dose, 540 mg every 8 hours, produced a toxic serum concentration. There was a decrease in serum quinine despite constant dosage, associated with a decrease in total serum bilirubin.

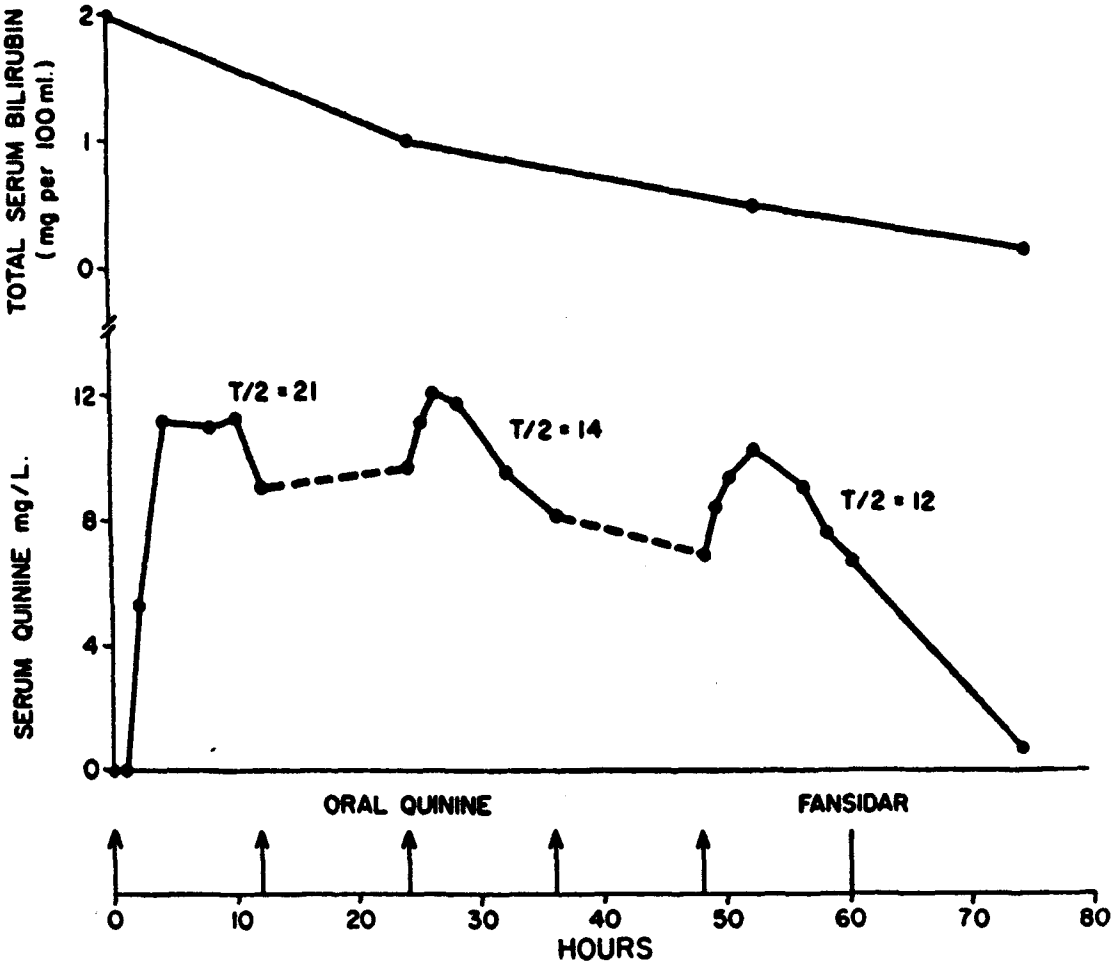

Figure 3. Clinical improvement and satisfactory serum quinine concentrations associated with 540 mg quinine every 12 hours. A decrease in the approximate half-life (T/2) of quinine was associated with decrease in total serum bilirubin.

Evaluation of a Direct Counting Technique for Malaria in Thailand

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Ampon Nanakorn, MT

OBJECTIVE: To evaluate a direct quantitative technique for the determination of parasite densities in malaria in rural hospitals in Thailand.

BACKGROUND: Various methods have been used for the direct quantification of malaria parasites but none has been widely used in tropical countries. The technique discussed in this report was introduced by Earle and Perez in 1932.

DESCRIPTION: A measured volume of blood (e.g. 0.005 ml) is spread evenly on a measured rectangle (e.g. 3 x 15 mm) on a slide. Thus the thickness of the film is known (0.11 mm). A grid is placed in one eyepiece of a binocular microscope and calibrated with a stage micrometer. Thus the volume of blood seen under one grid is known. For example, in the SEATO studies, about 1800 grids correspond to 1 cu. mm. of blood. Two types of staining are used. For outpatient screening 0.002 ml of blood are placed in the rectangle and stained with concentrated Giemsa (1:5 dilution) for five minutes. In patients admitted to the ward, 0.005 ml of blood are used and the thick film is stained with dilute Giemsa (1:50 dilution) for 30 minutes. Two rectangular thick films are prepared on inpatient slides. A circular thick film and a thin film are also prepared on all patients.

In this study the technicians were observed performing the parasite counts. There were five technicians on the study team. A stop watch was used to time the duration of the count. The following data were recorded for each determination of the parasite density. The name of the technician, the microscope used, the number of fields counted, the actual count obtained, the count per cu.mm. and the time elapsed in performing the count. A hand calculator was used for determining the parasite densities.

PROGRESS: The first factor assessed was the variability among technicians. Numerous duplicate counts on coded slides were performed by all the technicians. One technician counted with an accuracy of 3.4 per cent (Table 1) as determined on nine slides. The microscopes used were recalibrated frequently. No consistent difference in the two microscopes was observed.

The number of fields that need to be counted varies with the parasite count. For example, if the parasite count is 500,000 per cu.mm. there are about 250 parasites under each grid. The slide is scanned to find typical concentrations of parasites and with such a high count, a minimum of two fields have to be counted. At the other end of the scale a count of 500 requires the counting of at least 90 fields. Usually the number of fields counted is 2, 20, 90 or a larger number. Except for very low counts, about 200–500 parasites are counted. The accuracy of the technique is shown in Table 1. In this experiment the most accurate count was achieved in the patient with a parasite density of 10,000 (0.4 percent precision). The technique was less accurate with both high (4.1% precision), and low (11.1% precision) parasite densities. However the accuracy of the technique was satisfactory through all ranges of the parasite count. This technician read the nine slides twice in an average of 3.8 minutes per slide (range 2.1 to 6.3 minutes). Numerous other experiments have shown that only about four minutes is needed to make an accurate count.

SUMMARY: In rural hospitals in Thailand, a direct quantification technique has proved useful and accurate for the determination of parasite densities ir malaria.

Table 1. Duplicate Counts on 9 *Falciparum* Malaria Slides by One Observer Using The Direct Quantitative Technique

Slide	1st (a) Reading	2nd (b) Reading	Mean (c)	c—a	Precision $(\frac{c-a\%}{c})$
1	220220	202748	211484	8736	4.1
2	188188	182364	185276	2912	1.6
3	46592	47593	47092	501	1.1
4	29757	29302	29529	228	0.8
5	10920	10829	10874	46	0.4
6	2020	2220	2120	100	4.7
7	1660	1900	1780	120	6.7
8	400	500	450	50	11.1
9	10	20	—	—	—
	Mean 55529	53052	—	—	3.4*

* This figure is the reproducibility from the mean or "PRECISION" and is an index of the accuracy of the technique.

Treatment of Severe Malaria in Children

Principal Investigators: Anthony P. Hall, COL, MC
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Associate Investigator: Panya Sonkom, M.D.[1]

OBJECTIVE: To determine the optimum management of severe malaria in children.

BACKGROUND: During 1974 clearcut guidelines were developed at Trad for the management of severe malaria in adults. Specifically the maximum safe daily dose of quinine in adults was 20 mg/Kg. This confirms work performed in Malaysia 50 years ago but in the intervening period 30 mg/Kg has emerged as the recommended daily dose. Also at Trad in 1974 we determined that the maximum safe daily fluid intake was 1500 ml in adult patients with falciparum malaria. During this study period, it became apparent that the mortality rate of severe falciparum malaria in small children was high. The onset of convulsions is a frequent and grave complication in small children. The management of severe malaria in children has received little attention in the literature. Another problem is the difficulty often encountered in setting up or maintaining an intravenous infusion because of the small veins in infants.

DESCRIPTION: The children described in this study were treated at the Trad Provincial Hospital in Southeast Thailand. They were first evaluated in SEATO outpatient clinic and then admitted to the ward. Most of the patients were treated between April and July 1974 since there was an unusually high proportion of sick children presenting to the hospital during this interval. The use of the metering chamber (capacity 100 ml) was introduced so that precise volumes could be infused into the children.

PROGRESS: The most difficult problems in management occurred in small children and infants. Children above the age of 12 years could be treated similarly to adults except the dosage of any medication was best determined on a mg/kg basis. Therefore only the data on children aged 12 years and less are included in Table 1. Most of the children noted in the Table were very seriously ill on admission. It is difficult to infuse an effective but not toxic dose of quinine; therefore, details on the amount of quinine given as the first dose are shown in the Table. The recommended daily dose of quinine is not more than 30 mg/Kg/day or 10 mg/Kg every 8 hours. Most of the children received only one infusion every 24 hour interval because of the difficulty in initiating and maintaining infusions.

Early in the study the question arose whether 5 mg/Kg might not be a safer dose of quinine than 10 mg/Kg in children. However Case No. 630 and Case No. 684 were both admitted gravely ill and died a few hours after admission. They received smaller doses of quinine (5—6 mg/Kg). Whatever dose of intravenous quinine was used, some children improved and others deteriorated; however, in general, 5 mg/Kg appeared to be a safe and non—toxic dose of quinine. Some interesting case—histories are now given.

Cases 649—651. These three children, aged eight, five, and three years were siblings admitted at 1500 hours on 18 May 1974. Cases 649 and 651 were alert but toxic. Case No. 650, the three year old girl, was almost in coma. All three children received half—strength quinine, i.e. 0.5 mg/ml normal saline. Cases 649 and 650 received 5.0 mg/kg over about a four hour interval and 651 received 4 mg/Kg. All the patients improved and oral quinine therapy was commenced the next day. Case 649 received one tablet of quinine at 0600, 1400 and 2100 hours, case 650 received 1/2 tablet crushed in water at 0600 and 1400 hours and case 615 received 1/2 tablet at 0600, 1400 and 2100 hours. All the patients then received a dose of Fansidar. All three patients were radically cured although two had a mild *P. vivax* infection on day 28. None of these children was desperately ill, but all responded well to a half—dose of intravenous quinin

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1 Trad Provincial Hospital, Thailand

Case 686. This one year old child was admitted very breathless and crying. His pulse rate was 160 and respiratory rate 70. The liver and spleen were enlarged and hematocrit 24 per cent. He was drinking satisfactorily. To avoid pulmonary edema, a small volume (50 ml) containing 50 mg quinine was infused over a five hour interval. Oral quinine was then administered as a 1/2 tablet crushed in water every 12 hours, for a total of six doses. A 1/2 tablet of Fansidar was then given. The child made a satisfactory recovery.

DISCUSSION: Severe falciparum malaria in small children has a serious prognosis. Intravenous quinine therapy should be administered slowly in small amounts. The appropriate initial dose is usually 5 – 10 mg/kg administered as an intravenous infusion. Satisfactory results have been achieved by giving maintenance therapy as oral quinine when the patients' condition has improved. In infants, 1/2 tablet plain quinine crushed in water can easily be administered by the mother every 12 hours under close professional supervision. Repeated small doses of intravenous quinine should be administered if the child remains severely ill.

Table 1. Details of Initial Dose of Quinine in Children Aged 12 Years or Less with Severe Falciparum Malaria

Case Number	Age (yr)	Weight (Kg)	Parasite Count	Hematocrit (%)	First Dose Quinine (mg)	Volume Infused (ml)	Infusion Time (Hours)	Initial Dose (mg/kg)	Comment
609	7	?10	458000	38	180	180	5.0	? 18	Died
613	9	19	160000	23	180	180	4.5	9.5	
630	12	?25	334000	16	125	125	2.5	? 5.0	Died
640	2	10	1200000	11	117	235	12.0	12.0	Died
644	4	14	95000	24	82	165	5.0	6.0	
648	11	21	430000	31	100	200	6.0	5.0	
649	8	20	333000	33	95	190	4.0	5.0	
650	3	11	178000	29	55	110	3.7	5.0	
651	5	14	238000	30	55	110	3.7	4.0	
660	1.5	8	54000	18	40	80	5.0	5.0	
665	2	13	113000	20	45	90	4.0	4.0	Not enough Quinine
681	11	?	56000	12	350	?	?	? 17	Died
683	4	14	370000	32	150	250	5.0	11	
684	1	5	450000	12	31	63	6.0	6.0	Died
685	4	14	210000	26	100	100	3.0	7.0	
686	1	8.5	53000	24	50	50	5.0	6.0	
F27	5	18	65000	39	180	180	2.0	10.0	
F28	11	28	287000	41	210	210	3.0	7.5	
F31	8	20	256000	32	180	180	4.3	9.0	

A Study of Falciparum Malaria in Vietnam Using Serum Quinine Concentrations

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OBJECTIVE: To obtain further information on the relation between quinine dosage schedules and the serum level of the drug in patients treated for acute falciparum malaria.

BACKGROUND: Recent work in Thailand has suggested that quinine dosage every 12 hours is optimal therapy in falciparum malaria. Therefore this hypothesis was tested in a group of Vietnamese patients with falciparum malaria. The value of using serum quinine concentrations was also investigated.

DESCRIPTION: The study was performed on patients with malaria admitted to an infectious diseases ward of a general medical hospital in Saigon, South Vietnam. The patients were self-referred and presented with "fever" as their main complaint. A positive diagnosis of falciparum malaria was made when the thin smear on admission showed characteristic appearances of an acute *P. falciparum* infection.

Patients studied received an initial 600 mg dose of quinine given IV over a period of four hours in 500 ml of 5% dextrose in saline. Some patients continued to receive IV quinine at 12 hour intervals followed by a single dose of Fansidar (three tablets), while other patients were given oral quinine at 12 hour intervals followed by a single dose of Fansidar. Blood was drawn and the serum separated for quinine determinations at 0, 4, 8, 12 and 24 hours following the first quinine dose and also after the last quinine dose was administered.

The duration of fever and parasitemia were recorded and the severity of the malaria and any side-effects of the quinine were noted during the course of treatment.

PROGRESS: In November and December 1974, a total of 19 patients were studied. There were 12 male and 7 female patients with an age range of 13 to 63 years and a mean age of 28 years.

The mean duration of fever was 1.7 days and of parasitemia 3.2 days. The percentage parasite count on admission ranged from 0.1% to 36.0%. Nine patients had a count of 1.0% or less, three patients were between 1-3%, and three patients had between 3-6% parasitemia. High parasite counts of 36.0%, 35.0%, 12.8% and 12.0% were found in four patients.

Two patients died. One of these was a 42 year old man who developed tachypnea on the second hospital day after receiving four doses of IV quinine at 12 hour intervals. He had a petechial rash on his face and limbs, gum bleeding and coughed up bloody sputum. His lungs were clear on auscultation and he was not orthopneic. His parasite count was 0.4%. The other patient was a 63 year old man with cerebral malaria and a parasite count of 35%. He became anuric, failed to respond to fluid loading and mannitol with furosemide and died before dialysis could be initiated. He received a single IV dose of 600 mg quinine in a four hour infusion.

Several patients developed mild nausea and vomiting and two patients developed sinus bradycardia which returned to normal sinus rhythm on stopping the quinine.

Serum Quinine Levels: Preliminary results are available. Patient No. 1 was aged 13 and weighed 29 Kg. He received 1200 mg quinine (a double dose) as his first dose. The serum quinine peaked at 18 mg per L at four hours after dosing and then decreased rapidly with a half-life of about six hours. The patient received a total of four doses of intravenous quinine every 12 hours followed by a dose of pyrimethamine with sulfadoxine (Fansidar). Following the last dose of quinine his serum level peaked at 12 mg per cent. Despite the satisfactory levels of serum quinine obtained, he returned with a positive smear on day 15.

Patient No. 22 was anuric. His serum quinine concentration peaked at 17 mg per L following the first dose of quinine. Despite the anuria, the serum quinine then decreased rapidly with an approximate half-life of about eight hours. When the serum quinine levels have been completed, an overall analysis will be presented.

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